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Improved Estimates from the Schaefer Production Model: Theoretical Considerations

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SCHNUTE, J. 1977. Improved estimates from the Schaefer production model: theoretical considerations. J. Fish. Res. Board Can. 34: 583–603.

The Schaefer production model is converted to a form directly applicable to a data stream of annual fishing efforts and catches. The new version is also stochastic; that is, it allows for unpredictable influences on the fishery. A new method for estimating optimum effort and catch results from this analysis, as well as a way of measuring uncertainty in these estimates. Equations are given for predicting the next annual catch and assigning confidence limits to this prediction. Linear and nonlinear regressions are proposed for this analysis, and the relationship between them is rigorously demonstrated. The linear method leads to estimation formulas simple enough to be applied on a programmable pocket calculator.

Key words: Schaefer model, production model, stochastic model, management, fishing effort, catch per unit effort, maximum sustainable yield

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Le modèle de production de Schaefer est modifié de façon à être directement applicable à une série de données annuelles sur les efforts de pêche et sur les prises. La nouvelle version est également stochastique; c'est à dire qu'il y a place pour les facteurs imprévisibles influant sur les pêches. Il résulte de cette analyse une nouvelle méthode d'estimer les résultats optimaux relatifs aux efforts et aux captures ainsi qu'une façon de mesurer l'incertitude reliée à ces estimations. Des équations permettent de prévoir les captures de l'année suivante et d'en déterminer les limites de confiance. Des régressions linéaires et non linéaires sont proposées pour cette analyse, et la relation entre elles est rigoureusement démontrée. La méthode linéaire va de pair avec des formules d'estimation assez simples pour être résolues sur une calculette programmable.

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In simplest terms, a fishery is a resource in which boats go out to sea and come back with fish. Data most reflective of this process are records of the effort exerted and the resulting catch. Baranov (1925) made some of the earliest systematic uses of such data to argue that fisheries do not simply harvest excess fish, like interest on capital, but actually reduce the basic stock itself. He cited catch per unit effort before and after World War I to show how stocks could recover during a period of no fishing.

In a later independent work, Thompson and Bell (1934) demonstrated for the North Pacific halibut fishery that an unrestricted increase in effort ultimately leads to a reduced total catch. Schaefer (1954) carried this idea to mathematical fruition by applying a logistic growth model to estimate the maximum sustainable yield for yellowfin tuna in the eastern tropical Pacific. He revised his estimation procedure (Schaefer 1957) to produce the widely known three-parameter Schaefer model. More recently, Pella and Tomlinson (1969) used an expanded four-parameter model to monitor the yellowfin tuna fishery.

A model of effort and catch data for a fishery, a *production model*, may take several possible forms. Let $\bar{E}_n$ represent the average effort rate (in boat-days per year) and $\bar{C}_n$ the average catch rate (in pounds per year) for a fishery during year n, and suppose that these data are tabulated for a period of several years. Perhaps the simplest view of a fishery is that the effort determines the catch, that is,

$$(1) \quad \bar{C}_n = f(\bar{E}_n)$$

for some function f. However, a model of the form (1) assumes that the catch has no relation to the past history of the fishery. According to (1), for example, heavy fishing last year would not influence this year's catch. To allow for that possibility, (1) can be expanded to the form

$$(2) \quad \bar{C}_n = g(\bar{E}_n, \bar{C}_{n-1}, \bar{E}_{n-1})$$

for some function g.

Model (2) is *dynamic*; it represents the present state of the fishery in terms of its past state. By contrast, (1) ignores the past, an omission which is reasonable only if conditions in the fishery change slowly, and is called an *equilibrium* model.

Certainly (2) is more realistic than (1), but if any model (2) were really true, the problem of fishery management would be greatly simplified. It is never possible to predict the catch exactly, since there are always factors unaccounted for by the data. An extreme position in this regard was taken by Burkenroad (1948) who argued, counter to Thompson and Bell, that for North Pacific halibut the fishery was not the predominant factor in determining the fish stock. (See also Skud 1975.) If (2) is to be taken seriously, it must allow for the unexplained; for example, (2) could be replaced by

$$(3) \quad \bar{C}_n = h(\bar{E}_n, \bar{C}_{n-1}, \bar{E}_{n-1}, \epsilon_n)$$

for some function h, where ϵ_n is a random predictive error. Model (3) is not only dynamic, but *stochastic* as well.

Interestingly, equations of the types (2) or (3) do not appear in fisheries literature. Both the Schaefer model and the Pella and Tomlinson model are based on differential equations, that is, on processes continuous in time. However, (2) and (3) are *discrete-time* models which involve the transition from one year to the next.

The purpose of this paper is to recast the Schaefer model in the form (3), that is, as a dynamic, discrete-time, stochastic model. This new formulation has four principal advantages over the old one. First, equation (3) relates directly to the data in the hands of the manager, that is, a list of efforts and catches. Consequently, its method of application is more transparent. Second, because random errors are shown explicitly in (3), the parameter estimation scheme for the model is dictated unambiguously by the least-squares condition

$$(4) \quad \sum \epsilon_n^2 = \text{minimum}.$$

In fact, it appears that previous estimation methods are wrong and can be easily corrected. Third, again because (3) is stochastic, it is possible to estimate uncertainty (or variance) in the parameters,

as well as the parameters themselves. Similar remarks apply to uncertainty in the catch predicted by (3). Fourth, the new estimation formulas turn out (at least approximately) to be simple enough for a pocket calculator.

In practice, a manager using equations in this paper can quickly compute estimates of maximum sustainable yield and optimum effort from the history of effort and catch in his fishery. He can also calculate a prediction for the next year's catch. Finally, he can estimate the uncertainty in all these quantities.

An analysis very similar to the one in this paper can be used to convert the Pella and Tomlinson model to the form (3). However, for the sake of simplicity, only the Schaefer model is discussed here. Generalizations of this model will be investigated in a later work.

Assumptions of the Schaefer Model

To derive an equation of the form (1), (2), or (3), it is necessary to delineate precisely the assumptions, or axioms, of the Schaefer model. The version presented here differs slightly from that in earlier works. Changes have been made to achieve consistency in the dimensional units of all quantities appearing in the model and to reduce the number of axioms to three.

The Schaefer model contains one independent variable, time t, and four dependent variables, that is, four functions of time. These are the population biomass $P(t)$, the rate of fishing effort $E(t)$, the rate of catch $C(t)$, and the catch per unit effort $U(t)$. Finally there are three parameters: the natural growth rate r, the carrying capacity k, and the catchability coefficient q. These eight quantifiable ingredients of the model are listed in Table 1, together with typical units in which they might be measured.

To distinguish functions from constants, all functions are labeled with capital letters. For consistency, the rate of fishing effort is denoted by E instead of the usual f. It should perhaps be emphasized that E and C are rates. For example, fishing effort is commonly measured in boat-days. Thus the rate of effort might be measured in boat-days per year. Notice that 365 boat-days/yr equals 1 boat, so that another measure of E might be, simply, boats.

The axioms of the model consist of three equations relating the eight quantities in Table 1:

$$(5) \quad \frac{dP}{dt} = rP\left(1 - \frac{P}{k}\right) - C,$$

$$(6) \quad C = qEP,$$

$$(7) \quad U = qP.$$

The first equation states that the total growth rate of the population biomass equals its natural logistic growth rate minus the catch rate. The second equation (6) says that the catch rate is directly proportional to the effort rate and to the available biomass; the constant of proportionality is q, the catchability coefficient. Finally (7) says that the catch per unit effort is itself proportional to the biomass alone. This idea is closely related to (6); in fact (6) and (7) imply that *if* $E \neq 0$, then

$$(8) \quad U = \frac{C}{E}.$$

It might seem that (8) should be the axiom instead of (7). However, (8) is meaningful only when $E \neq 0$, and (7) is meaningful even when $E = 0$. It follows from (7) that the catch per unit effort, in general, is not zero even if the effort itself is zero. Therefore, (7) suggests the correct interpretation for U, namely that U is a potential catch per unit effort. This potential is actualized only when fishing takes place, that is, when $E \neq 0$.

Notice, incidentally, from (8) that U is the ratio of catch rate to effort rate. In taking the ratio, the rates cancel so that U is dimensionally catch per unit effort, as it should be.

It is also instructive to consider the units of the parameters. For example, k is measured in pounds and represents the weight that a naturally restricted fish stock would ultimately reach in the absence of fishing. In addition, r has the units years^{-1}, so that $1/r$ is a number of years. Explicitly, $1/r$ is the

TABLE 1. Mathematical components of the model.

Classification	Notation	Name	Typical units
Independent variable	t	time	yr
Functions	$P(t)$	population biomass	lb
	$E(t)$	fishing effort rate	boat-days/yr
	$C(t)$	catch rate	lb/yr
	$U(t)$	catch per unit effort	lb/(boat-day)
Parameters	r	natural growth rate	$(\text{yr})^{-1}$
	k	carrying capacity	lb
	q	catchability coefficient	$(\text{boat-day})^{-1}$

time required for an unrestricted, unfished population to increase by the factor $e = 2.71 \ldots$. Similarly, $1/q$ is the number of boat-days of fishing required to reduce a population by the factor $1/e = 0.367 \ldots$ in the absence of natural mortality or growth.

Mathematically, (5)–(7) can be used as follows. Suppose that the parameters r, k, and q are known, that the population biomass $P(t_0)$ is known at some time t_0, and that the effort rate $E(t)$ is known for $t \geq t_0$. Then the three equations (5)–(7) can be used to compute the three functions $P(t)$, $C(t)$, and $U(t)$ for $t \geq t_0$. Therefore, the model uses effort to predict, among other things, catch per unit effort. In fact, (5)–(7) imply that

$$(9) \quad \frac{dU}{dt} = rU\left(1 - \frac{U}{qk}\right) - qEU.$$

Notice that (9) is an equation for U, once E is known. In words, (9) predicts catch per unit effort from effort. This is a matter of central concern to the manager. In practice he can ignore the population biomass and focus directly on his potential catch. The fishery is a system which is tested by applying an effort rate E and detecting a catch per unit effort U. In this sense the single equation (9) encapsulates the whole purpose of the three equations (5)–(7).

Averages

The Schaefer model (5)–(7) is a continuous-time model. Converting it to the form (1) or (2) involves averaging, or integrating, it over some time period. Suppose that $F(t)$ is a function of time and that $\tau > 0$ is a period of time. Typically τ is 1 yr, although in some cases it might be the mean generation time for the species. Define

$$F_n = F(n\tau),$$

$$\bar{F}_n = \frac{1}{\tau}\int_{n\tau}^{(n+1)\tau} F(t)\,dt.$$

This definition can be applied to any of the four functions P, E, C, and U. For example, P_n represents the population biomass at the start of year n, and $\bar{P}_n$ represents the average biomass during year n. Here "average" means "time average."

It follows from this definition that $\bar{E}_n$ and $\bar{C}_n$ are the usual total effort per year and catch per year normally recorded by biologists and managers. This is because, for example, the integral of the effort rate is the total effort. Notice, however, that $\bar{E}_n$ and $\bar{C}_n$ have the same dimensional units as E and C, respectively. This results from the factor $1/\tau$, where τ is a time, in the definition of average.

All authors to date, for example Baranov (1925), Thompson and Bell (1934), Schaefer (1957), Pella and Tomlinson (1969), and Skud (1975), calculate the annual catch per unit effort from the ratio $\bar{C}_n/\bar{E}_n$. Unfortunately, it is not necessarily true that

$$(10) \quad \bar{U}_n = \frac{\bar{C}_n}{\bar{E}_n}.$$

The difficulty is that $\overline{U}_n$ is the average of the ratio C/E. This does not always equal the ratio of averages $\overline{C}_n/\overline{E}_n$. However, an average ratio does equal the ratio of averages if the denominator is constant. In other words, (10) is true if the rate of fishing is constant during each year, that is, if

$$(11)\quad E(t) = \overline{E}_n \quad n\tau \leq t < (n+1)\tau,$$

for each n. When (11) is not true (10) may be only approximate. However, in many cases (10) is the best approximation available, because for parts of the year there may be no fishing at all. During such times the ratio C/E is simply not measured or known; consequently, the average ratio C/E can't be exactly computed.

In this paper, as in all previous work, the approximation (10) will often be used. The condition (11) gives at least one circumstance in the fishery for which (10) is exact.

Traditional Estimation

As a first application of the definitions just given, it is useful to consider briefly a traditional method for estimating the parameters r, k, and q. If (9) is rewritten as

$$\frac{1}{U}\frac{dU}{dt} = r - qE - \frac{r}{qk}U,$$

and this equation is integrated from $n\tau$ to $(n+1)\tau$, it follows that

$$(12)\quad \ln\left(\frac{U_{n+1}}{U_n}\right) = r\tau - q\tau\overline{E}_n - \frac{r\tau}{qk}\overline{U}_n.$$

Here the variable $\ln(U_{n+1}/U_n)$ is expressed as a linear function of $\overline{E}_n$ and $\overline{U}_n$. Consequently, (12) suggests a linear regression of one variable on two. Such a regression gives three parameters that can be used to find r, k, and q.

Unfortunately, although typical fishery data include $\overline{E}_n$ and $\overline{U}_n$, the quantity $\ln(U_{n+1}/U_n)$ usually is not known because it involves instantaneous values at the start of each year. Few biologists trust fishery data taken over a very short time. However, one possible approximation for U_n might be

$$(13)\quad U_n \cong \tfrac{1}{2}(\overline{U}_n + \overline{U}_{n-1});$$

that is, the catch per unit effort U at the start of year n is approximately equal to (denoted "$\cong$") the average of the two annual averages for U in the years just following and just preceding the first day of year n. *If* (13) is exactly true for each n, then (12) becomes

$$(14)\quad \ln\left(\frac{\overline{U}_{n+1} + \overline{U}_n}{\overline{U}_n + \overline{U}_{n-1}}\right) = r\tau - q\tau\overline{E}_n - \frac{r\tau}{qk}\overline{U}_n.$$

Equation (14) can be taken as the basis for using annual fishery data to estimate three parameters by the linear regression of one variable on two. Indeed, this method is closely related to that of Schaefer (1957), Fox (1970), and (in a slightly altered context) Walter (1973).

Yet there is something disturbing about (14). If (14) were true, then $\overline{U}_{n+1}$ could be predicted *without knowing* $\overline{E}_{n+1}$. In other words, it would be possible to predict next year's catch per unit effort without specifying next year's anticipated effort. This conclusion not only contradicts almost all fisheries biology, it even contradicts equations (5)–(7) from which (14) supposedly came.

To obtain a more realistic equation than (14), add (12) for year n to (12) for year $n+1$, and divide by 2. The result is

$$(15)\quad \ln\left(\sqrt{\frac{U_{n+1}U_{n+2}}{U_nU_{n+1}}}\right) = r\tau - q\tau\left(\frac{\overline{E}_n + \overline{E}_{n+1}}{2}\right) - \frac{r\tau}{qk}\left(\frac{\overline{U}_n + \overline{U}_{n+1}}{2}\right).$$

Notice that (15), like (12), is an exact consequence of (5)–(7). Next, instead of (13), suppose that

$$(16)\quad \overline{U}_n \cong \sqrt{U_nU_{n+1}},$$

that is, the annual average catch per unit effort is, roughly, the geometric mean of its values at the beginning and end of each year. If (16) is exact, then (15) becomes

$$(17)\quad \ln\left(\frac{\overline{U}_{n+1}}{\overline{U}_n}\right) = r\tau - q\tau\left(\frac{\overline{E}_n + \overline{E}_{n+1}}{2}\right) - \frac{r\tau}{qk}\left(\frac{\overline{U}_n + \overline{U}_{n+1}}{2}\right).$$

Like (14), (17) is an equation that can be used to obtain three parameters by regressing one variable on two. Unlike (14), if (17) is solved for $\overline{U}_{n+1}$, the result involves $\overline{E}_{n+1}$; therefore, (17) is logically preferable to (14). However, a price has been paid for this improvement. Since $\overline{U}_{n+1}$ is the predicted quantity, it is not clear in (17) whether to regress the variable $\ln(\overline{U}_{n+1}/\overline{U}_n)$ or the variable $(\overline{U}_n + \overline{U}_{n+1})/2$ on the other two variables. This difficulty points to the need for a stochastic model (3). In such a model, the least squares condition (4) dictates the correct regression.

Equilibrium

In examining a fishery model, it is usually instructive to look first at the equilibrium case. Biologically, this gives a reasonable approximation when the fishery conditions change slowly. Mathematically, the model equations are much simpler at equilibrium.

For the Schaefer model, two distinct types of equilibrium are relevant. Define the system (5)–(7) to be in *continuous equilibrium* if the population biomass $P(t)$ is a nonzero constant P for all time. Also, define *periodic equilibrium* for (5)–(7) by the condition that P_n, the biomass at the start of each year, is the same nonzero constant every year.

In continuous equilibrium, then, the biomass is always the same. This might happen, for example, if a constant level of fishing just balances population growth. By contrast, in periodic equilibrium the population may vary, but it always returns to the same value at the start of each year. Such a situation might occur, for example, if each year the stock is fished for several months and then allowed to recover to its initial level for the start of the next fishing season.

It is possible to deduce from (5)–(7) that when P is a nonzero constant, the remaining functions E, C, and U are also constant. In fact, (5)–(7) imply that at continuous equilibrium

$$(18)\quad P = k\left(1 - \frac{q}{r}E\right),$$

$$(19)\quad U = qk\left(1 - \frac{q}{r}E\right),$$

$$(20)\quad C = qkE\left(1 - \frac{q}{r}E\right).$$

Equations (18)–(20) express the constant biomass, catch per unit effort, and catch rate which result from a fixed effort rate E at continuous equilibrium.

Notice in (20) that C is a parabolic function of E; it increases for small E but decreases for large E. This prediction corresponds to Schaefer's version of the claim by Thompson and Bell (1934) that too great a fishing effort ultimately reduces the total catch. The maximum value of C in (20), that is, the *maximum equilibrium catch rate* (or maximum sustainable catch rate) is given by

$$(21)\quad C_e = \frac{rk}{4}.$$

This catch rate is achieved when the sustained effort rate, catch per unit effort, and biomass are

$$(22)\quad E_e = \frac{r}{2q},\quad U_e = \frac{qk}{2},\quad P_e = \frac{k}{2},$$

respectively. Notice, incidentally, from (21) and (22) that

$$C_e = E_e U_e; \tag{23}$$

this is consistent with (8).

The relations (22) suggest another possible set of parameters for the Schaefer model. Originally, the model was formulated with the three parameters r, k, and q. Alternatively, one can use the three optimum condition parameters E_e, U_e, and P_e. The transition from the first set to the second is given by (22); the transition back is given by

$$r = \frac{2E_e U_e}{P_e}, \quad k = 2P_e, \quad q = \frac{U_e}{P_e}. \tag{24}$$

At periodic equilibrium, the equations (18)–(20) are replaced by

$$\bar{P}_n = k\left(1 - \frac{q}{r}\bar{E}_n\right), \tag{25}$$

$$\bar{U}_n = qk\left(1 - \frac{q}{r}\bar{E}_n\right), \tag{26}$$

$$\bar{C}_n = qk\bar{E}_n\left(1 - \frac{q}{r}\bar{E}_n\right). \tag{27}$$

The key detail in proving these equations is that

$$\ln\left(\frac{U_{n+1}}{U_n}\right) = \ln\left(\frac{qP_{n+1}}{qP_n}\right) = \ln 1 = 0$$

at periodic equilibrium. This fact in (12) gives (26). Then (25) follows from (7). Finally (27), which is really only approximate, follows from the approximation (10).

Although the two sets of equations (18)–(20) and (25)–(27) look very similar, there is an important difference between them. In (18)–(20) E, P, U, and C are constant for all time. However, in (25)–(27) $\bar{E}_n$, $\bar{P}_n$, $\bar{U}_n$, and $\bar{C}_n$ can be different for different values of n. In his original paper, Schaefer (1954) calls (26) the "line of equilibrium conditions." However, he obtains (26) by assuming that

$$\frac{dU}{dt} = 0,$$

that is, by assuming *continuous* equilibrium. No one seems to have pointed out the distinction between (19) and (26) or the fact that (26) follows from much weaker assumptions. *All that is needed for the fishery to conform to (26) is that the population should return to the same biomass level at the start of each year.*

Equation (27) is the Schaefer form of (1), an annual prediction of catch from effort. Notice that, although (27) appears to contain three parameters, it actually depends only on two, qk and q/r. In fact, with the aid of (22), equations (26) and (27) can be written

$$\bar{U}_n = U_e\left(2 - \frac{\bar{E}_n}{E_e}\right), \tag{28}$$

$$\bar{C}_n = U_e\bar{E}_n\left(2 - \frac{\bar{E}_n}{E_e}\right), \tag{29}$$

respectively. It follows that (29) is a two-parameter equilibrium model of the form (1) whose parameters might, for example, be estimated from the linear regression (28) of $\bar{U}_n$ on $\bar{E}_n$. Furthermore, these two parameters are the optimum fishing effort E_e and the resulting catch per unit effort U_e. The optimum catch C_e can, in turn, be found from (23). The only parameter of the Schaefer

model not found by this method is P_e, the optimum population biomass. However, it might well be argued that this is irrelevant since, regardless of the population level, the critical question for the manager is how to harvest the most fish on a sustained basis.

The real difficulty with (29) is that described for (1) in the introduction: (29) does not relate this year's catch to last year's catch and effort. Consequently, (29) cannot allow for the consequences of the past. To do so requires a dynamic equation of the form (2).

Dynamics

In deriving an equation of the form (2) from (5)–(7), it is necessary to make some assumption about how the fishing effort is spread out in time during the year. One possibility is described by (11); the rate of fishing may be constant during each year (although it may vary from year to year). An important advantage to (11) is that it is consistent with (10), the usual rule for calculating the annual catch per unit effort. If (11) is true, then a series of manipulations on (5)–(7) can be used to show that

$$(30)\quad \frac{\exp\left(\frac{r\tau}{qk}\,\bar{U}_n\right) - 1}{\exp\left(\frac{r\tau}{qk}\,\bar{U}_{n-1}\right) - 1} = \frac{r - q\bar{E}_{n-1}}{r - q\bar{E}_n}\,\frac{\exp((r - q\bar{E}_n)\tau) - 1}{\exp((r - q\bar{E}_{n-1})\tau) - 1}\,\exp\left(\left(r - q\bar{E}_{n-1} - \frac{r}{qk}\,\bar{U}_{n-1}\right)\tau\right).$$

The proof of (30) is somewhat lengthy and is deferred to Appendix A. The procedure relies heavily on equation (12).

Although the biological significance of (30) may not be obvious, one key aspect of this equation is reasonably clear: it is possible to solve (30) for $\bar{U}_n$. More precisely, $\bar{U}_n$ is seen to be a function of $\bar{U}_{n-1}$, $\bar{E}_{n-1}$, and $\bar{E}_n$. Therefore, (30) almost has the desired form (2); in fact, from (10) and (30) it follows that

$$(31)\quad \bar{C}_n = \frac{qk}{r\tau}\,\bar{E}_n \ln\left[1 + \frac{r - q\bar{E}_{n-1}}{r - q\bar{E}_n}\,\frac{\exp((r - q\bar{E}_n)\tau) - 1}{1 - \exp(-(r - q\bar{E}_{n-1})\tau)}\left(1 - \exp\left(-\frac{r\tau}{qk}\,\frac{\bar{C}_{n-1}}{\bar{E}_{n-1}}\right)\right)\right].$$

This is the Schaefer form of the dynamic equation (2).

In summary, equations (30) and (31) are logical consequences of equations (5)–(7) and (11). Furthermore, (30) and (31) are the dynamic parallels of the equilibrium equations (28) and (29), respectively. In particular, (30) is the dynamic counterpart to Schaefer's "line of equilibrium." Finally, (29) and (31) are the Schaefer versions of the production equations (1) and (2).

Although (28) and (29) involve only two parameters, (30) and (31) properly contain all three parameters r, k, and q (or E_e, U_e, and P_e). The problem of estimating these parameters is taken up in the section on stochastics.

Biological Interpretation

The biological significance of the discrete equilibrium version of the Schaefer model is quite straightforward. According to (26), $\bar{E}_n$ and $\bar{U}_n$ are linearly related; as the annual effort goes up, the annual catch per unit effort goes down. By (25), this results from a population decrease due to heavier fishing.

One difficulty with (26) is that it predicts a negative catch per unit effort $\bar{U}_n$ when the effort is large enough, that is, when

$$\bar{E}_n > \frac{r}{q} = 2E_e.$$

This, of course, is biologically impossible. It is better to interpret (26) as predicting that the maximum allowable catch rate is $2E_e$. At that level of fishing and beyond, the population will be driven to extinction.

To interpret the dynamic equation (30), define the two dimensionless variables

$$(32)\quad v_n = \exp((r - q\bar{E}_n)\tau),\ w_n = \exp\left(\frac{r\tau}{qk}\,\bar{U}_n\right).$$

Notice that v_n depends on $\bar{E}_n$. Essentially, v_n is an adjusted effort rate; a bigger $\bar{E}_n$ gives a smaller v_n. In general v_n is positive, but it approaches zero as the effort rate becomes infinite. Similarly, w_n is an adjusted catch per unit effort which increases as $\bar{U}_n$ increases; w_n approaches 1 as $\bar{U}_n$ approaches zero, that is, as the stock approaches extinction.

With the substitutions (32), (30) can be written in the form

$$(33)\quad w_n - 1 = (w_{n-1} - 1)\frac{\ln v_{n-1}/(v_{n-1} - 1)}{\ln v_n/(v_n - 1)}\frac{v_{n-1}}{w_{n-1}}.$$

To interpret (33), which is equivalent to (30), it is necessary to interpret the three quantities (i) $w_n - 1$, (ii) $\ln v_n/(v_n - 1)$, and (iii) v_n/w_n. Since w_n is a measure of catch per unit effort, and since w_n equals 1 at extinction, it is appropriate to call $w_n - 1$ the *extinction distance* in year n. A small extinction distance means that the stock is close to extinction. Unlike ordinary distances, the extinction distance is dimensionless.

To interpret the second quantity, notice that

$$\frac{\ln v_n}{v_n - 1} = \frac{(r - q\bar{E}_n)\tau}{\exp((r - q\bar{E}_n)\tau) - 1}$$

It can be shown that this is an increasing function of the effort rate $\bar{E}_n$. Therefore, the second quantity simply represents a dimensionless recalibration of the effort rate. It is called the *scaled effort* for year n.

The third quantity

$$\frac{v_n}{w_n} = \exp\left((r - q\bar{E}_n - \frac{r}{qk}\,\bar{U}_n)\tau\right)$$

equals 1 when

$$r - q\bar{E}_n - \frac{r}{qk}\,\bar{U}_n = 0.$$

This is equivalent to the equilibrium equation (26). In other words, the ratio v_n/w_n equals 1 when the effort rate in year n is at just the right level to hold the stock fixed. For this reason, v_n/w_n is called the *equilibrium factor*. If the equilibrium factor is <1, then the effort is large for the stock size and will tend to drive it down. Conversely, if the equilibrium factor is >1, then the effort is small for the stock size, and the population will tend to increase.

With the terminology just defined, it is possible to interpret (33) biologically. Let year n be "this year." Then (33) predicts this year's catch per unit effort by predicting this year's extinction distance. Explicitly, (33) states that this year's extinction distance equals last year's multiplied by two correction factors: (a) the ratio of last year's to this year's scaled efforts and (b) last year's equilibrium factor. In other words, (33) prescribes an increase or decrease in the extinction distance which depends on a ratio of scaled efforts and an equilibrium factor. The extinction distance tends to increase, for example, if this year's scaled effort is less than last year's. Biologically this conforms to the idea that a reduced effort allows the stock to increase. Similarly, an increase in extinction distance is favored by an equilibrium factor bigger than 1 last year, that is, by low fishing last year in relation to stock size.

Mathematically, (33) is a so-called *recursion equation* for predicting a sequence of w_n's from a sequence of v_n's (or vice versa). It is possible to make a rigorous study of the properties of (33) and, consequently, (30). In biological language, these mathematical results imply the following four basic properties of the dynamic equation (30):

Property I. If $\bar{E}_n$ is held for many years at a fixed level larger than $2E_e$, then the stock declines steadily to extinction.

Property II. If $\bar{E}_n$ is held for many years at a fixed level smaller than $2E_e$, but large for the original stock size, then the stock declines steadily to a new equilibrium size.

Property III. If $\bar{E}_n$ is held for many years at a fixed level small for the original stock size, then the stock increases steadily to a new equilibrium size.

Property IV. To hold the stock size constant over a period of many years, the manager must either hold the effort fixed at the equilibrium level or else follow a policy of alternate overfishing and underfishing which converges to the equilibrium level.

It is emphasized that these properties are logical consequences of (30), which itself follows from (5)–(7) and (11). Proofs appear in Appendix B.

These properties illustrate the way in which the dynamic equation (30) generalizes the equilibrium equation (26). Like (26), (30) includes the concepts of equilibrium and extinction. However, (30) improves (26) because it describes the dynamic process of approach to equilibrium or extinction. Also, unlike (26), (30) always predicts a positive catch per unit effort.

Stochastics

So far, essentially two arguments have been made for using (30) to model a fishery. First, (30) is a logical consequence of the classical Schaefer model. Second, (30) exhibits general properties which appear to be biologically reasonable. However, in the real world Properties I–IV never hold exactly, and it is seemingly impossible to predict next year's catch exactly.

If (30) is to model the world realistically, it must allow for predictive error. None of the assumptions leading to (30) allow for such error, and, consequently, (30) is deterministic. The question is, where should the error term appear? It seems wise to follow the often-cited principle of William of Occam (1280–1349), not to multiply hypotheses except out of necessity. In this case, an error term is necessary; but, for a start, it should be included in the simplest way possible.

Equation (30) predicts the extinction distance as a product. Equivalently, the logarithm of the extinction distance is given as a sum of logarithms. The simplest way to allow for error in a sum is to add a normally distributed random variable. If (30) is modified with these ideas in mind, it becomes

$$(34)\quad \frac{\exp\left(\frac{r\tau}{qk}\,\overline{U}_n\right) - 1}{\exp\left(\frac{r\tau}{qk}\,\overline{U}_{n-1}\right) - 1} = \frac{r - q\bar{E}_{n-1}}{r - q\bar{E}_n}\,\frac{\exp((r - q\bar{E}_n)\tau) - 1}{\exp((r - qE_{n-1})\tau) - 1}\exp\left(\left(r - q\bar{E}_{n-1} - \frac{r}{qk}\,\overline{U}_{n-1}\right)\tau\right)\exp(\sigma\epsilon_n),$$

where ϵ_n is a normally distributed random variable with mean 0 and variance 1, and σ is a dimensionless parameter equal to the standard error of prediction. It is assumed that ϵ_m and ϵ_n are independent for different years $m \neq n$.

Equation (34) is a stochastic version of (30). Biologically, it states that a ratio of successive extinction distances equals the product of a ratio of scaled efforts, an equilibrium factor, and a random factor. By taking the logarithm of each side of (34), one sees that $\sigma\epsilon_n$ is an additive error in predicting the variable

$$\ln\left(\exp\left(\frac{r\tau}{qk}\,\overline{U}_n\right) - 1\right) = \ln(w_n - 1),$$

that is, the logarithm of the extinction distance. Notice that (34) is a four-parameter model. It includes r, k, and q as before; in addition, it contains σ, the standard error in predicting $\ln(w_n - 1)$. As σ approaches zero, (34) approaches the deterministic equation (30).

From (34) and (10), it follows that

$$(35)\quad \overline{C}_n = \frac{qk}{r\tau}\bar{E}_n \ln\left[1 + \frac{r - q\bar{E}_{n-1}}{r - q\bar{E}_n}\,\frac{\exp((r - q\bar{E}_n)\tau) - 1}{1 - \exp(-(r - q\bar{E}_{n-1})\tau)}\left(1 - \exp\left(-\frac{r\tau}{qk}\,\frac{\overline{C}_{n-1}}{\bar{E}_{n-1}}\right)\right)\exp(\sigma\epsilon_n)\right].$$

This is a stochastic form of (31); it describes at least one possible function h for a model equation (3). There are, of course, many possible ways to include an error term in (31). But (35) is one of the simplest because it comes about from one of the simplest modifications to the related equation (30).

The three pairs of equations (28)–(29), (30)–(31), and (34)–(35) form a hierarchy. The first pair is a two-parameter equilibrium model, the second is a three parameter dynamic model, and the third is a four-parameter dynamic and stochastic model. Furthermore, (29), (31), and (35) are versions of (1), (2), and (3), respectively, appropriate to the Schaefer model.

Least Squares Estimation

Suppose that a sequence of effort and catch data is known for a series of years $n = 0, 1, \ldots, N$. To use this historical information in planning for the future with the Schaefer model, it is necessary to estimate the parameters in (34). The maximum likelihood estimates for r, k, and q are those which minimize the sum of squares of the (normally distributed) predictive errors,

$$S = \sum_{n=1}^{N} \sigma^2 \epsilon_n^2.$$

If (34) is solved for $\sigma\epsilon_n$, then S can be represented explicitly in terms of r, k, and q; that is,

$$S(r, k, q) = \sum_{n=1}^{N} \left\{ \ln\left[\frac{\exp\left(\frac{r\tau}{qk}\overline{U}_n\right) - 1}{\exp\left(\frac{r\tau}{qk}\overline{U}_{n-1}\right) - 1} \frac{r - q\overline{E}_n}{r - q\overline{E}_{n-1}} \frac{\exp((r - q\overline{E}_{n-1})\tau) - 1}{\exp((r - q\overline{E}_n)\tau) - 1} \right] - \left(r - q\overline{E}_{n-1} - \frac{r}{qk}\overline{U}_{n-1}\right)\tau \right\}^2.$$

The desired estimates $\hat{r}$, $\hat{k}$, and $\hat{q}$, then, are those numbers which solve the problem

(36) $S(\hat{r}, \hat{k}, \hat{q}) = \text{minimum},$

for a given data sequence $\overline{E}_0, \overline{U}_0, \overline{E}_1, \overline{U}_1, \ldots, \overline{E}_N, \overline{U}_N$.

Unfortunately, problem (36) can be technically difficult to solve. Since S is a complicated function of the parameters, (36) necessarily involves the use of a computer algorithm for nonlinear parameter estimation. The serious user of Schaefer's model inevitably encounters this difficulty.

However, there is a remarkably simple alternative to (36) if an approximation is allowed. It turns out that, if a and b are two numbers close to each other, then

$$(37) \quad \frac{a}{b}\frac{e^b - 1}{e^a - 1} e^{(a-b)/2} \cong 1.$$

When a and b are small, (37) is especially good. For example, if $(a, b) = (.4, .6)$, the left side of (37) is 1.008. If a and b both lie between .2 and 1, then (37) is never wrong by more than 4%. The same is true for a and b between 1 and 1.4. Incidentally, (37) is not new to fisheries literature. It was used earlier, in slightly different form, by Pope (1972) for estimating age-specific rates of fishing mortality on a year-class.

The approximation (37) can be applied to the left side of (34) with

$$a = \frac{r\tau}{qk}\overline{U}_{n-1}, \quad b = \frac{r\tau}{qk}\overline{U}_n$$

and to the right side with

$$a = (r - q\overline{E}_{n-1})\tau, \; b = (r - q\overline{E}_n)\tau.$$

Notice that a and b are close to each other in both cases if conditions change slowly in the fishery. In view of this, (34) and (37) suggest the approximation

$$\frac{U_n}{U_{n-1}}\exp\left(\frac{r\tau}{qk}(\bar{U}_n - \bar{U}_{n-1})/2\right)$$

$$\cong \exp(-q(\bar{E}_n - \bar{E}_{n-1})\tau/2)\exp\left(\left(r - q\bar{E}_{n-1} - \frac{r}{qk}\bar{U}_{n-1}\right)\tau\right)\exp(\sigma\epsilon_n),$$

that is,

$$(38)\quad \ln\left(\frac{\bar{U}_n}{\bar{U}_{n-1}}\right) \cong r\tau - q\tau\left(\frac{\bar{E}_{n-1} + \bar{E}_n}{2}\right) - \frac{r\tau}{qk}\left(\frac{\bar{U}_{n-1} + \bar{U}_n}{2}\right) + \sigma\epsilon_n.$$

Equation (38) is an approximate form of (34) *which is also a stochastic version of (17)*, with n replaced by $n - 1$. Consequently, it appears that the early attempt (17) to estimate parameters is quite reasonable, provided that an error term is added as in (38). In fact, if

$$S_1(r, k, q) = \sum_{n=1}^{N}\left[\ln\left(\frac{\bar{U}_n}{\bar{U}_{n-1}}\right) - r\tau + q\tau\left(\frac{\bar{E}_{n-1} + \bar{E}_n}{2}\right) + \frac{r\tau}{qk}\left(\frac{\bar{U}_{n-1} + \bar{U}_n}{2}\right)\right]^2,$$

then (38) shows that S_1 is a good approximation to S, at least when conditions change slowly in the fishery. Therefore, an approximate solution to the problem (36) is the solution to

$$(39)\quad S_1(\hat{r}, \hat{k}, \hat{q}) = \text{minimum}.$$

This greatly simplifies the nonlinear estimation (36) because (39) is an ordinary *linear* regression which can readily be used to produce estimates for $r\tau$, $q\tau$, and $r\tau/qk$; then, in turn, estimates for r, k, and q can be deduced. (Recall that τ is a known fixed time period, such as 1 yr.)

In retrospect, two facts emerge. First, (17) is indeed a reasonable basis for regression because it approximates the dynamic equation (30) whose stochastic counterpart is (34). Second, $\ln(\bar{U}_n/\bar{U}_{n-1})$ is the correct variable to regress on the other two; this follows from the position of the error term in (38).

From the theory of linear regression, it is possible to solve problem (39) explicitly. Some extra notation helps simplify the results. Define the quantities

$$(40)\quad \begin{aligned} x_n &= \tfrac{1}{2}(\bar{E}_{n-1} + \bar{E}_n)\\ y_n &= \tfrac{1}{2}(\bar{U}_{n-1} + \bar{U}_n)\\ z_n &= \ln(\bar{U}_n/\bar{U}_{n-1}). \end{aligned}$$

Let $\bar{x}$ be the usual mean

$$\bar{x} = \frac{1}{N}\sum_{n=1}^{N} x_n,$$

with similar definitions for $\bar{y}$ and $\bar{z}$. Also, let

$$S_x = \sum_{n=1}^{N}(x_n - \bar{x})^2,$$

$$S_{xy} = \sum_{n=1}^{N}(x_n - \bar{x})(y_n - \bar{y}),$$

with similar meanings for S_y, S_z, S_{xz}, and S_{yz}. Then the solution to (39) turns out to be

$$\hat{r} = \frac{1}{\tau}\frac{\bar{x}(S_{xy}S_{yz} - S_yS_{xz}) + \bar{y}(S_{xy}S_{xz} - S_xS_{yz}) + \bar{z}(S_xS_y - S_{xy}^2)}{S_xS_y - S_{xy}^2}$$

$$(41)\quad \hat{k} = \tau^2\hat{r}\frac{(S_xS_y - S_{xy}^2)^2}{(S_{xy}S_{yz} - S_yS_{xz})(S_{xy}S_{xz} - S_xS_{yz})}$$

$$\hat{q} = \frac{1}{\tau}\frac{S_{xy}S_{yz} - S_yS_{xz}}{S_xS_y - S_{xy}^2}.$$

An abbreviated proof of (41) appears in Appendix C.

Notice that equations (40) and (41) give estimates for the three Schaefer parameters directly from fishery data. These results can be plugged into (22)–(23) to give estimates $\hat{E}_e$, $\hat{U}_e$, $\hat{C}_e$, and $\hat{P}_e$. In particular, then, (41) provides the key to an explicit calculation of the conditions for maximum sustainable yield.

It should be emphasized that (41) solves the *linear* regression (39). This is only an approximation to the nonlinear regression (36). If a computer is available, refined estimates $\hat{r}$, $\hat{k}$, and $\hat{q}$ can be found which solve (36). Since all known methods for doing this are iterative, (41) is still useful as a good first guess to begin the iterative process.

From (40), x_n has the units of E, y_n has the units of U, and z_n is dimensionless. The interested reader can use these facts to verify that the formulas (41) are dimensionally correct; that is, $\hat{r}$, $\hat{k}$, and $\hat{q}$ have the units shown in Table 1.

Variance

An error analysis should serve two purposes. First, it should give a measure of uncertainty in the parameter estimates $\hat{E}_e$, $\hat{U}_e$, and $\hat{P}_e$, or, equivalently, $\hat{r}$, $\hat{k}$, and $\hat{q}$. Typically this takes the form of a three-dimensional confidence region around the point $(\hat{E}_e, \hat{U}_e, \hat{P}_e)$. The analysis should predict that, with a given probability, the true parameter values (E_e, U_e, P_e) lie within such a confidence region around the estimated values $(\hat{E}_e, \hat{U}_e, \hat{P}_e)$. Second, an error analysis should give a measure of uncertainty in prediction. Once the estimates $\hat{r}$, $\hat{k}$, and $\hat{q}$ are known, based on the years $0, 1, \ldots, N$, then (31) can be used to predict the catch for the next year $N + 1$. However, this prediction suffers from an error due to the use of estimates $(\hat{r}, \hat{k}, \hat{q})$ instead of true parameter values (r, k, q). Also, as (35) shows, there is an additional peredictive error $\sigma\epsilon_{N+1}$ due to the stochastic nature of the fishery. All these errors should be accounted for in an analysis of uncertainty in prediction.

The estimates (41) follow by the usual linear regression arguments from (38). In the notation of (40), (38) reads

$$z_n \cong r\tau - q\tau x_n - \frac{r\tau}{qk} y_n + \sigma\epsilon_n. \tag{42}$$

It is tempting in (42) to suppose that the error lies in z_n alone. Unfortunately, this is not the case. Recall that (42), or (38), approximates (34) in which the predicted variable is $\overline{U}_n$. Since both y_n and z_n involve $\overline{U}_n$, it follows that y_n and z_n are influenced by the predictive error ϵ_n.

To see precisely how the error enters y_n and z_n, notice from (40) that

$$y_n = \tfrac{1}{2}\overline{U}_{n-1}(1 + e^{z_n}). \tag{43}$$

Therefore (42) can also be written in the form

$$z_n \cong r\tau - q\tau x_n - \frac{r\tau}{2qk}\overline{U}_{n-1}(1 + e^{z_n}) + \sigma\epsilon_n. \tag{44}$$

In (44) it is correct to say that the error influences z_n alone. Unfortunately, it is only possible to solve (44) for z_n numerically. At best, z_n is a complicated function of ϵ_n. By implication from (43), so is y_n.

In summary, (42) falsely suggests that z_n is a simple linear function of ϵ_n. Instead, (43) and (44) show that y_n and z_n are complicated functions of ϵ_n. This fact does not affect the estimation procedure (41) that results from the usual least squares condition (39). But it does affect variance estimates. The usual theory does not apply because the parameter estimates (41) are much more complicated functions of the error than usual.

Fortunately, there is at least an approximate way out of the complex error analysis implied by (43) and (44). If conditions change reasonably slowly in the fishery, then $\overline{U}_n$ is close to $\overline{U}_{n-1}$. From (40), this implies that

$$y_n \cong \overline{U}_{n-1}, z_n \cong 0. \tag{45}$$

Consequently, it is reasonable to perform an approximate error analysis assuming that z_n is small

and y_n is error free. This approximation corresponds to assuming that the fishery is nearly, but not exactly, in periodic equilibrium.

With the aid of approximations (42) and (45), three results from linear regression analysis can be carried over approximately to the stochastic model (34)–(35). The first result is that an unbiased estimate for the parameter σ^2 is

$$(46)\quad \hat{\sigma}^2 = \frac{1}{N-3} S_1(\hat{r}, \hat{k}, \hat{q}).$$

Second, if $F_\alpha(3, N-3)$ is the upper α probability point of the Fisher distribution with 3 and $N-3$ degrees of freedom, then

$$(47)\quad S_1(r,k,q) - S_1(\hat{r}, \hat{k}, \hat{q}) \leq 3\hat{\sigma}^2 F_\alpha(3, N-3)$$

with probability α. Third, let $\hat{\epsilon}_{N+1}$ be the predictive error in (34) or (35) which results both from stochastic error and from the use of parameter estimates $(\hat{r}, \hat{q}, \hat{k}, \hat{\sigma})$ rather than true values. In other words, let $\hat{\epsilon}_{N+1}$ be defined by the equation

$$\frac{\exp\left(\frac{\hat{r}\tau}{\hat{q}\hat{k}} \overline{U}_{N+1}\right) - 1}{\exp\left(\frac{\hat{r}\tau}{\hat{q}\hat{k}} \overline{U}_N\right) - 1} = \frac{\hat{r} - \hat{q}\overline{E}_N}{\hat{r} - \hat{q}\overline{E}_{N+1}} \frac{\exp((\hat{r} - \hat{q}\overline{E}_{N+1})\tau) - 1}{\exp((\hat{r} - \hat{q}\overline{E}_N)\tau) - 1} \exp\left(\left(\hat{r} - \hat{q}\overline{E}_N - \frac{\hat{r}}{\hat{q}\hat{k}} \overline{U}_N\right)\tau\right) \exp(\hat{\sigma}\hat{\epsilon}_{N+1}).$$

Then, if $t_{\alpha/2}(N-3)$ is the upper $\alpha/2$ probability point of Student's t distribution with $N-3$ degrees of freedom, it follows that

$$(48)\quad R_N t_{\alpha/2}(N-3) < \hat{\epsilon}_{n+1} < R_N t_{\alpha/2}(N-3)$$

with probability α, where

$$R_N = \sqrt{\frac{N+1}{N} + \frac{(x_{N+1} - \bar{x})^2 S_y - 2(x_{N+1} - \bar{x})(\overline{U}_N - \bar{y}) S_{xy} + (\overline{U}_N - \bar{y})^2 S_x}{S_x S_y - S_{xy}^2}}.$$

In this expression the quantities $\bar{x}$, $\bar{y}$, S_x, and S_y are calculated as before from $x_1, y_1, \ldots, x_N, y_N$ without using x_{N+1} and y_{N+1}.

It is emphasized that the three statistical results (46)–(48) are rigorous consequences of the stochastic Schaefer model (34) and the two approximations (42) and (45). Detailed proofs are too lengthy to include in this paper; however, brief plausibility arguments are presented in Appendix D. Full proofs may be obtained from the author.

The results (46)–(48) can be used to describe uncertainties in estimation and prediction, as required by an error analysis. Of course, (46) simply gives an estimate for σ^2, the stochastic variance of the model. This estimate is used in (47) which is a condition giving confidence limits on the estimates $\hat{r}$, d, and $\hat{q}$. For a given confidence level α, (47) requires that the sum of squares $S_1(r, k, q)$ must not exceed its minimum value $S_1(\hat{r}, \hat{k}, \hat{q})$ by more than a certain amount. This can happen only if the true values (r, k, q) are restricted to a certain region around the estimated values $(\hat{r}, \hat{k}, \hat{q})$. That region is the α-level confidence region for (r, k, q).

Since (r, k, q) can be converted to (E_e, U_e, P_e) by (24), (47) can be rewritten as a condition on (E_e, U_e, P_e), namely,

$$(49)\quad S_1(E_e, U_e, P_e) - S_1(\hat{E}_e, \hat{U}_e, \hat{P}_e) \leq 3\hat{\sigma}^2 F_\alpha(3, N-3)$$

with probability α. In particular, the confidence region (49) restricts the managerial parameters E_e and U_e. Also, the extreme values of

$$C_e = E_e U_e$$

in this region give limits to the optimum catch.

The condition (48) sets confidence limits on the predictive error ϵ_{N+1} in (35) when parameter estimates are used instead of true values. The actual prediction for $\bar{C}_{N+1}$ and its upper and lower α-level confidence limits are obtained by substituting

$$(\hat{r}, \hat{k}, \hat{q}, \hat{\sigma}, 0),$$

$$(50) \quad (\hat{r}, \hat{k}, \hat{q}, \hat{\sigma}, R_N t_{\alpha/2}(N-3)),$$

$$(\hat{r}, \hat{k}, \hat{q}, \hat{\sigma}, -R_N t_{\alpha/2}(N-3)),$$

respectively, for $(r, k, q, \sigma, \epsilon_{N+1})$ in (35) when $n = N+1$. Notice that the predicted catch $\bar{C}_{N+1}$ corresponds to setting $\epsilon_{N+1} = 0$, while upper and lower confidence limits for $\bar{C}_{N+1}$ correspond to setting ϵ_{n+1} at the extreme values of $\hat{\epsilon}_{N+1}$ in (48).

It is important for this calculation that all quantities appearing in the substitutions (50) are known at the time of prediction. In particular, the estimates $\hat{r}$, $\hat{k}$, $\hat{q}$, and $\hat{\sigma}$ are determined by past data for the years $0, 1, \ldots, N$. Similarly, R_N depends on these data as well as the projected effort $\bar{E}_{n+1}$. (This occurs in x_{N+1}).

As a rough gauge of the model's overall success, consider the quantity

$$(51) \quad I = \frac{S_1(\hat{r}, \hat{k}, \hat{q})}{S_z}.$$

This represents the fraction of unexplained variance in the predicted variable z; if $\hat{r}$, $\hat{k}$, and $\hat{q}$ solve (39), then it can be shown that $0 \leq I \leq 1$. If I is near 0, the model works reasonably well. However, larger values of I, especially near 1, suggest that the model works poorly; that is, past and present efforts are poor predictors of catch. Accordingly, I is called the *failure index*. It provides an objective measure for the validity of Burkenroad's (1948) claim that the fishery is not the predominant factor influencing the fish stock.

The discussion of variance so far has consistently assumed that the estimates $\hat{r}$, $\hat{k}$, and $\hat{q}$ are given by (41), that is, by the solution to the linear regression problem (39). In case those estimates come from the nonlinear regression (36), then all preceding remarks apply, except that S_1 in (46), (47), (49), and (51) should be replaced by S. In particular, the failure index becomes

$$I = \frac{S(\hat{r}, \hat{k}, \hat{q})}{S_z},$$

a quantity which, it can be shown, lies between 0 and 1 when $\hat{r}$, $\hat{k}$, and $\hat{q}$ solve (36).

The logical cornerstones of the entire discussion on estimation and variance are equations (34), (35), (42), and (45). First, (34) and (35) set out the basic equations of the Schaefer model in its new form. Then (42), which approximates (34), can be used to linearize the process of parameter estimation. Finally, the additional approximation (45) linearizes the error analysis necessary to assign variance limits on the estimates and on the model's predictions. Notice that approximations (42) and (45) are closely related; both apply when conditions in the fishery change slowly near periodic equilibrium.

Summary for the User

Input to the Schaefer model is a data stream of average effort rates $\bar{E}_n$ and catches per unit effort $\bar{U}_n$ for $n = 0, 1, \ldots, N$. These data can be used to estimate the optimum sustainable catch rate C_e, effort rate E_e, catch per unit effort U_e, and population size P_e. In addition, approximate confidence limits on these parameters can be determined for a given confidence level α. Finally, the model can be used to predict next year's catch $\bar{C}_{N+1}$ from past data and next year's effort $\bar{E}_{N+1}$, and approximate α-level confidence limits can be assigned to the prediction.

Two related types of calculation can be used. One is an approximate linear regression which gives answers in a single step. Here the expressions for the desired estimates are simple enough to be programmed on a pocket calculator. Alternatively, a nonlinear regression gives more precise results but requires an iterative procedure on a computer. Estimates from the linear regression can be used to initiate this process.

The linear method begins with the calculation of x_n, y_n, and z_n ($n = 1, \ldots, N$), according to (40). Then $\hat{r}$, $\hat{k}$, and $\hat{q}$ are computed by (41). From these three estimates, the corresponding estimates $\hat{E}_e$, $\hat{U}_e$, $\hat{C}_e$, and $\hat{P}_e$ can be found by (22) and (23). Also, an estimate for σ^2 is given by (46), where $S_1(\hat{r}, \hat{k}, \hat{q})$ can be directly evaluated from the estimates just determined along with the original data. The inequality (49) is a condition for determining α-level confidence limits on $\hat{E}_e$, $\hat{U}_e$, and $\hat{P}_e$, and, in turn, $\hat{C}_e$.

Once $\hat{r}$, $\hat{k}$, $\hat{q}$, and $\hat{\sigma}$ are known, they can be used to predict next year's catch $\bar{C}_{N+1}$. This prediction and its upper and lower α-level confidence limits are given, respectively, by making the three substitutions (50) in (35) when $n = N + 1$. Finally, the failure index (51) gives a rough measure of the model's overall success. Notice that only six equations, or sets of equations, are involved in this entire process: (40)–(41), (22)–(23), (46), (49), (35) with (50), and (51).

The nonlinear method proceeds similarly, except that (36) is solved iteratively for $\hat{r}$, $\hat{k}$, and $\hat{q}$. The estimates (41) can be taken as a first approximation. Once the final estimates are determined, the user proceeds as above, except that S_1 is replaced by S in (46), (49), and (51).

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Appendix A. Proof of (30)

For notational simplicity, define the two quantities

$$\text{(A1)} \quad \begin{aligned} \alpha_n &= r - q\bar{E}_n, \\ \beta &= \frac{r}{qk}. \end{aligned}$$

From (11), it follows that

$$\alpha_n = r - qE$$

on the time interval

$$\text{(A2)} \quad n\tau \leq t < (n+1)\tau.$$

Therefore, during this interval, (9) can be written as

(A3) $$\frac{1}{U}\frac{dU}{dt} = \alpha_n - \beta U.$$

Notice that

(A4) $$\frac{d}{dt}\left(\frac{\exp(\alpha_n t)}{U}\right) = \frac{\alpha_n \exp(\alpha_n t)}{U} - \frac{\exp(\alpha_n t)}{U^2}\frac{dU}{dt}.$$

Substituting (A3) in (A4) shows that, during the interval (A2),

(A5) $$\frac{d}{dt}\left(\frac{\exp(\alpha_n t)}{U}\right) = \beta \exp(\alpha_n t).$$

An integration of (A5) over the internal (A2) gives

(A6) $$\frac{\exp(\alpha_n t)}{U_{n+1}} - \frac{1}{U_n} = \frac{\beta}{\alpha_n}(\exp(\alpha_n t) - 1),$$

where a factor $\exp(n\alpha_n\tau)$ has been cancelled from both sides.

Equation (A6) involves the two quantities U_n and U_{n+1}. The same remark applies to (12) which, in the notation of (A1), reads

(A7) $$\ln\left(\frac{U_{n+1}}{U_n}\right) = \alpha_n\tau - \beta\tau\overline{U}_n.$$

It is possible to solve the two equations (A6) and (A7) simultaneously for U_n and U_{n+1}. In particular,

(A8) $$U_n = \frac{\alpha_n}{\beta}\frac{\exp(\beta\tau\overline{U}_n) - 1}{\exp(\alpha_n\tau) - 1}.$$

Similarly, by repeating all steps leading to (A8) with n replaced by $n + 1$, it follows that

(A9) $$U_{n+1} = \frac{\alpha_{n+1}}{\beta}\frac{\exp(\beta\tau\overline{U}_{n+1}) - 1}{\exp(\alpha_{n+1}\tau) - 1}.$$

Equations (A7), (A8), and (A9) can be used to prove (30). First, divide (A9) by (A8) to obtain an expression for U_{n+1}/U_n. Then substitute this expression in (A7). Finally, (30) follows from the resulting form of (A7) after a little algebraic manipulation. This last step, of course, involves the substitutions (A1).

Appendix B. Proof of Properties I–IV

The desired properties hinge on two mathematical theorems about equation (33). These are:

THEOREM 1. *Suppose that $v_n = v$ for every n, $w_0 > 1$, and $v > 0$. Then*

(i) *if $v < 1$, then w_n converges steadily downward* to 1;
(ii) *if $1 \leq v < w_0$, then w_n converges steadily downward* to v;
(iii) *if $v = w_0$, then $w_n = v$ for every n;*
(iv) *if $w_0 < v$, then w_n converges steadily upward to v.*

THEOREM 2. *Suppose that $w_n = w$ for every n, where $w > 1$. Then*

(i) *if $v_0 = w$, then $v_n = w$ for every n;*
(ii) *if $1 < v_0 < w$, then v_n converges steadily to w by alternating between values below and above w.*

To interpret Theorem 1, notice that "$v_n = v$ *for every* n" means that the effort rate $\overline{E}_n$ remains the same for every year n. Also, "$w_0 > 1$" means simply that the population is not extinct. The condition $v > 0$ is compatible with (32) in which v_n appears as an exponential. Notice from (32) that if $v_n = v < 1$, then

$$r - q\overline{E}_n < 0,$$

that is,

$$\bar{E}_n > \frac{r}{q} = 2E_e.$$

In other words, "$v < 1$" implies that the effort rate exceeds the maximum allowable level. If $1 < v < w_0$, then the effort rate is allowable, but still large for the initial population size because the initial equilibrium factor v/w_0 is <1. Similarly, if $w_0 < v$, then the effort rate is small for the initial population. The phrase "w_n *converges downward to* 1" implies that the population declines steadily to extinction. Similarly, if w_n converges downward (upward) to v, then the population moves steadily to a level appropriate to the effort rate because the equilibrium factor v/w_n tends toward 1.

In view of these interpretations, the conclusions (i), (ii), and (iv) of Theorem 1 imply Properties I, II, and III, respectively. Also (iii) shows that if the effort rate is just right for the initial population, then the population remains fixed. Finally, with the aid of interpretations similar to those described in the last paragraph, Theorem 2 implies Property IV. Notice the hypothesis "$1 < v_0 < w$" in (ii). This requires that the initial overfishing effort $\bar{E}_0$ must not exceed $2E_e$.

To prove Theorem 1, notice that if $v_n = v$ for every n, then (33) implies

$$\text{(B1)} \quad w_n = 1 + v\left(1 - \frac{1}{w_{n-1}}\right).$$

Subtracting (B1) from a second version of (B1) with n replaced by $n + 1$ gives

$$\text{(B2)} \quad w_{n+1} - w_n = \frac{v}{w_{n-1}w_n}(w_n - w_{n-1}).$$

Also, another way to write (B1) is

$$\text{(B3)} \quad w_n - v = \frac{1}{w_{n-1}}(w_{n-1} - v).$$

Taking absolute values in (B3) gives

$$\text{(B4)} \quad |w_n - v| = \frac{1}{|w_{n-1}|}|w_{n-1} - v|.$$

Since $v > 0$ and $w_0 > 1$, (B1) can be used to prove inductively that $w_n > 1$ for every n. Therefore, in (B2) the factor $v/(w_{n-1}\, w_n)$ is always positive, and so, by induction, $w_{n+1} - w_n$ always has the same sign. It follows that the sequence $[w_n]$ is monotone. Similarly, (B3) shows that $[w_n]$ lies entirely above or below v. Also, since $1/w_{n-1}$ is a positive number <1, (B4) shows that the distance $|w_n - v|$ is decreasing.

In summary, $[w_n]$ is a monotone bounded sequence which lies above 1, remains on the same side of the number v, and moves steadily toward v. Therefore, the sequence has a limit $w \geq 1$. From (B1) it follows that w satisfies the equation

$$w = 1 + v\left(1 - \frac{1}{w}\right),$$

that is

$$\text{(B5)} \quad w^2 - (1 + v)w + v = 0.$$

The quadratic equation (B5) has only the two solutions $w = 1$ or $w = v$.

If $v < 1$, then the limit w must be 1 since $w \geq 1$. Also, since w_n gets steadily closer to v, the sequence $[w_n]$ moves steadily downward. This proves (i). If $1 < v < w_o$, then the limit w can only be v, since $[w_n]$ remains on the same side of v. This proves (ii). If $w_0 = v$, then $w_n = v$ from (B1) by induction. This proves (iii). If $1 < w_0 < v$, then w must be v because w_n moves steadily toward v which is a number >1. This proves (iv) and completes the proof of Theorem 1.

The proof of Theorem 2 hinges on the use of the three functions

$$f(v) = \frac{v - 1}{\ln v},$$

$$g(v) = \frac{f(v)}{v},$$

$$h(v) = \frac{f(v)}{\sqrt{v}}.$$

It can be shown that $f(v)$ increases steadily for $v > 0$, $g(v)$ decreases steadily for $v > 0$, and $h(v)$ increases steadily for $v > 1$. Also, these functions are all positive for $v > 0$. When $w_n = w$ for every n, (33) becomes

$$\text{(B6)} \quad f(v_n) = f(v_{n-1})\frac{w}{v_{n-1}},$$

or, equivalently,

$$\text{(B7)} \quad \frac{f(v_n)}{f(w)} = \frac{g(v_{n-1})}{g(w)}.$$

To prove (i), notice from (B6) that if $v_0 = w$, then $f(v_1) = f(w)$. Since f is strictly increasing, it follows that $v_1 = w$. Similarly, by induction, $v_n = w$ for every n.

The first step in proving (ii) is to show that

$$\text{(B8)} \quad w < v_1 < \frac{w^2}{v_0}.$$

Since $v_0 < w$ and g is decreasing, it follows that $g(v_0) > g(w)$. Therefore, by (B7), $f(v_1) > f(w)$. Since f is increasing, this implies that $v_1 > w$ and proves the left half of (B8). To prove the right half, notice from the definition of h that

$$\text{(B9)} \quad f\left(\frac{w^2}{v_0}\right) = h\left(\frac{w^2}{v_0}\right)\frac{w}{\sqrt{v_0}}.$$

Also, from (B6) it follows that

$$\text{(B10)} \quad f(v_1) = h(v_0)\frac{w}{\sqrt{v_0}}.$$

Since $v_0 < w$, it follows that ${v_0}^2 < w^2$ and $v_0 < w^2/v_0$. Therefore, since $h(v)$ is increasing for $v > 1$, and since $v_0 > 1$, (B9) and (B10) imply that

$$f(v_1) < f\left(\frac{w^2}{v_0}\right).$$

This inequality implies the right half of (B8), since f is increasing.

By repeating the steps leading to (B8), it is possible to show inductively that

$$\text{(B11)} \quad 1 < v_0 < \frac{w^2}{v_1} < v_2 < \frac{w^2}{v_3} < v_4 < \ldots < w < \ldots < v_3 < \frac{w^2}{v_2} < v_1 < \frac{w^2}{v_0} < w^2.$$

Part of the proof of (B11) involves the logical equivalence of certain pairs of inequalities. For example, $1 < v_0$ implies $w^2/v_0 < w^2$. Similarly $v_1 < w^2/v_0$ implies $v_0 < w^2/v_1$.

The inequalities (B11) show that the sequences $[v_n]_{\text{neven}}$ and $[v_n]_{\text{nodd}}$ have limits; call these limits v_a and v_b respectively. Again from (B11)

(B12) $1 < v_a \leq w \leq v_b$.

Furthermore, (B6) shows that

(B13) $f(v_a) = f(v_b)\dfrac{w}{v_b}$,

(B14) $f(v_b) = f(v_a)\dfrac{w}{v_a}$.

After dividing (B13) by (B14) and simplifying, it follows that

$$\frac{[f(v_a)]^2}{v_a} = \frac{[f(v_b)]^2}{v_b}.$$

Therefore, $h(v_a) = h(v_b)$ and $v_a = v_b$, since $h(v)$ is strictly increasing for $v \geq 1$. Also, from (B12) $v_a = w$. This completes the proof of Theorem 2.

Appendix C. Proof of (41)

The sum of squares S_1 can be written in the form

$$S_1(a, b, c) = \sum_{n=1}^{N} (z_n - a + bx_n + cy_n)^2,$$

where

(C1) $a = r\tau, \quad b = q\tau, \quad c = \dfrac{r\tau}{qk}$.

The three parameters a, b, and c form an equivalent set to r, k, and q. Equations (C1) give the relationship in one direction; the inverse equations are

(C2) $r = \dfrac{a}{\tau}, \quad k = \dfrac{a\tau}{bc}, \quad q = \dfrac{b}{\tau}$.

Estimates for a, b, and c are obtained as usual by minimizing S_1, that is, by finding the point $(\hat{a}, \hat{b}, \hat{c})$ where the three derivatives of S_1 with respect to a, b, and c vanish. These three derivative equations turn out to be equivalent to the system

$$\begin{aligned} &\hat{a} - \hat{b}\bar{x} - \hat{c}\bar{y} = \bar{z}, \\ (C3)\quad &\hat{b}S_x + \hat{c}S_{xy} = -S_{xz}, \\ &\hat{b}S_{xy} + \hat{c}S_y = -S_{yz}. \end{aligned}$$

Solving (C3) and substituting the result in (C2) gives (41).

Appendix D. Intuitive Reasons for (46)–(48)

Since S_1 approximates S, it follows from the definition of S that

$$\frac{1}{\sigma^2} S_1(r, k, q) \cong \sum_{n=1}^{N} \epsilon_n{}^2.$$

Consequently, because a sum of squares of independent normally distributed variables with mean 0 and variance 1 is chi-square,

(D1) $\dfrac{1}{\sigma^2} S_1(r, k, q) \sim \chi^2(N)$,

approximately. Here "$\sim$" means "is distributed as," and $\chi^2(N)$ is the chi-square distribution with N degrees of freedom. Similarly,

$$\text{(D2)}\quad \frac{1}{\sigma^2} S_1(\hat{r}, \hat{k}, \hat{q}) \sim \chi^2(N - 3),$$

with a loss of three degrees of freedom because S_1 is evaluated at estimates rather than true values of the three parameters r, k, and q. From (D2) it follows that the expected value of $S_1(\hat{r}, \hat{k}, \hat{q})/\sigma^2$ is $N - 3$. Therefore, an unbiased estimate for σ^2 is given by (46).

Subtracting (D2) from (D1) gives

$$\text{(D3)}\quad \frac{1}{\sigma^2}[S_1(r, k, q) - S_1(\hat{r}, \hat{k}, \hat{q})] \sim \chi^2(3).$$

Next, after dividing (D3) by (D2),

$$\text{(D4)}\quad \frac{N - 3}{3} \frac{S_1(r, k, q) - S_1(\hat{r}, \hat{k}, \hat{q})}{S_1(\hat{r}, \hat{k}, \hat{q})} \sim F(3, N - 3),$$

since the ratio of two independent chi-square distributions is F-distributed. Notice that the statistics (D3) and (D4) are always positive (as chi-square and F statistics should be) because S_1 is positive and has its minimum value at $(\hat{r}, \hat{k}, \hat{q})$. Furthermore, substituting (46) in (D4) shows that (47) is true with probability α.

From (46) and (D2), it follows that

$$\frac{(N - 3)\hat{\sigma}^2}{\sigma^2} \sim \chi^2(N - 3).$$

Therefore,

$$\text{(D5)}\quad \frac{\sigma\epsilon_{N+1}}{\hat{\sigma}} \sim t(N - 3),$$

since the ratio of a normal variable to the square root of an independent chi-square variable (divided by its degrees of freedom) is t-distributed. In (D5), the error $\sigma\epsilon_{N+1}$ is the total predictive error that occurs in (34) or (35) when true parameters (r, k, q) are used. If these true values are replaced with estimates $(\hat{r}, \hat{k}, \hat{q})$, then, by definition of $\hat{\epsilon}_{N+1}$, the total predictive error is $\hat{\sigma}\hat{\epsilon}_{N+1}$. In this case (D5) becomes

$$\text{(D6)}\quad \frac{\hat{\epsilon}_{N+1}}{R_N} \sim t(N - 3),$$

where $R_N > 1$ is a correction factor that approaches 1 as N becomes large. In passing from (D5) to (D6), $\hat{\sigma}$ cancels from the numerator and denominator. By (D6), (48) holds with probability α.

Fishery and Reproductive Cycle of the Red Sea Urchin, *Strongylocentrotus franciscanus*, in British Columbia

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BERNARD, F. R. 1977. Fishery and reproductive cycle of the red sea urchin, *Strongylocentrotus franciscanus*, in British Columbia. J. Fish. Res. Board Can. 34: 604–610.

Gametogenesis and gonadal index undergo an annual cycle with minima in September and maxima in May for a population of red urchins, *Strongylocentrotus franciscanus*, from southwestern Vancouver Island. Postspawning recovery is rapid and there are no significant differences between male and female gonadal indices, but a small gonad to body-size correlation is evident. Gonadal index is not the product of gametogenesis, but is correlated to glycogen accumulation which may reach 13% gonad total weight. The fishing season for roe in southern British Columbia extends from October to April with several weeks variability depending on local conditions and weather. Yields constitute up to 25% total drained weight.

Key words: urchins, fishery, gametogenesis, glycogen, hermaphroditism, sex ratio

BERNARD, F. R. 1977. Fishery and reproductive cycle of the red sea urchin, *Strongylocentrotus franciscanus*, in British Columbia. J. Fish. Res. Board Can. 34: 604–610.

Chez une population d'oursins, *Strongylocentrotus franciscanus*, du sud-ouest de l'île Vancouver, la gamétogénèse et l'indice des gonades ont un cycle annuel comportant un minimum en septembre et un maximum en mai. La récupération après la ponte est rapide, et il n'y aucune différence significative entre les indices de gonades des mâles et des femelles, mais une certaine relation est évidente entre la grosseur des gonades et celle du corps. L'indice des gonades n'est pas le produit de la gamétogénèse, mais est relié à l'accumulation du glycogène qui peut atteindre 13% du poids total des gonades. La saison de la pêche pour les gonades dans le sud de cette province s'étend d'octobre à avril, mais peut varier de plusieurs semaines dépendant des conditions locales et du temps. Les gonades constituent alors jusqu'à 25% du poids sec total.

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THE large red sea urchin, *Strongylocentrotus franciscanus* (Agassiz), occurs throughout the shallow subtidal rocky foreshore and deeper waters of British Columbia. Japanese demand for sea urchin roe has resulted in several attempts to establish a fishery. During 1970–72 urchins were harvested from Clayoquot Sound on the southwestern coast of Vancouver Island, and the roe from approximately 220,000 urchins exported each season. Two years of operations depleted the local fishable stock and harvesting was moved to the southern part of the Strait of Georgia until this area was closed to commercial fishing.

Surveys of population structure and gonadal yield of British Columbia red sea urchins have appeared in preliminary form (Bernard and Miller 1973a, b; Miller 1975; Kramer and Nordin 1975). This paper discusses the fishery and interprets the annual gametogenic cycle for a red urchin population from a small area of southwestern Vancouver Island and summarizes the results of several years of surveys throughout British Columbia.

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The Fishery

Two of three shallow water sea urchins occurring in British Columbia are of potential commercial significance. The small green urchin, *S. droebachiensis* (Müller), similar to the species utilized in Europe, though abundant, is too small for economic roe recovery, so commercial operations are entirely based on the red urchin.

The roe may represent up to 25% of the total drained weight and is closely correlated to population density and food availability. Unlike other roe products, such as herring roe, quality and yield in urchins are not dependent on the gametogenic cycle but are the result of nutrient accumulation. Gonads in advanced gametogenesis are not acceptable due to their softened texture

and leaking gametes. Peak condition occurs shortly after the postspawning recovery and declines throughout the period of ripening of the gametes; harvest ceases when the breeding condition is reached. In southern British Columbia the harvest period is from October to April but may vary a month or more depending on local conditions and the seasonality of the large benthic algae on which urchins feed. Average yields from fishable urchins range from 40 to 150 g/urchin and do not greatly vary through the season for a particular location. As harvesting and processing costs remain constant, it is essential that only high-yielding populations are fished. A successful operation requires the scouting and selection of potential sites prior to harvest and balancing population density with gonad yield. Unfortunately these criteria are inversely correlated; scattered urchins adjacent to algal beds display the maximum, and densely aggregated, easily fished, urchins the minimum gonad development. Expense and time in diving make 5 urchins/m^2 the minimal fishable density, but the working of very dense colonies ($\approx$30 urchins/m^2) is not profitable.

Effective management of this resource will require more information on the significance of clustering to reproductive biology and the ability of red urchins to recolonize harvested areas. In common with many other northern invertebrates, recruitment into established populations and the establishment of new colonies are infrequent, though the release of gametes and the appearance of early larvae in the plankton is an annual event.

Reproductive cycle

Materials and Methods

Gametogenesis and gonadal yield of urchins collected in a 10 m^2 area 6 m below LWST between Amphitrite Point and Francis Island on the southwest coast of Vancouver Island, B.C. (48°55.4′N, 125°31.6′W) were monitored from monthly samples of 50 individuals of commercial size (test diam > 100 mm). The test diameter was recorded, the shell bisected, and the perivisceral fluid drained. The drained total weight was recorded; then the gonads were removed, stripped of adhering mesentery, and weighed after blotting. The gonad color was noted and the gonadal index computed as a percentage of the total drained weight. A vertical section from the center of one gonad from each individual was fixed in buffered Formol–saline and microscope slides prepared stained in hematoxylin–eosin. The glycogen content of the gonad was determined by the anthrone reaction (Seifter et al. 1950) after extraction in 10% trichloroacetic acid.

The reproductive cycle was followed by recording the relative frequencies of cytologically defined stages in gametogenesis in a cross section from the wall to the central lumen of a randomly selected acinus. Each of two operators categorized 200 cells from each individual and the results were expressed as percentages.

Results

The study population occupied a small bay with a large boulder and fragmented stone substrate and abundant macroalgae. The average density was 7 urchins/m^2 (range 4–10), and size distribution was virtually unimodal with small urchins making up < 2% of the population. The average test diameter of individuals sampled was 125 mm with a SD 16 mm.

The sexes are separate and easily determined by gonad color; females are yellow and males yellow–orange when in prime condition, but during starvation resorption of the gametes occurs and both sexes become dark brown. There is no evidence of alternating sexuality, but one functional hermaphrodite was identified among 1317 individuals from Clayoquot Sound. The specimen bore three ovaries and two adjacent ovotestes. In the majority of locations sampled sex distribution approached equality, but some populations were strongly biased in favor of one sex. (Fleming Pass $N = 400$: 0.24 ♂; Gabriola Island $N = 111$: 0.59 ♂).

The gonadal index undergoes an annual cycle (Fig. 1 *upper*). The minimum is immediately after spawning in September and the maximum in May. The post spawning recovery is rapid with 80% of the maximal levels achieved by October. There is no significant statistical difference between male and female gonadal indices ($P = >0.05$) during the fishing season. There is a small difference (<5%) the balance of the year due to the more abrupt male spawning and recovery phase. A body size correlation is evident; the ratio of gonad to body size increases until $\approx$ 95 mm test diam is reached; thereafter the ratio decreases slightly with increasing size.

In both sexes there is a small increase in gonadal index during early stages of gametogenesis, and just prior to breeding the gonad increases 2–4% in weight with no parallel increase in glycogen. Histological examinations reveal that the gametes hereto closely packed in the acinal lumina are now separate, and as the gonads are soft, the gametes leak out.

Gonadal index is correlated to the accumulation of glycogen, which may reach 13% of the total gonad dry weight (Fig. 1 *lower*). Monthly unpaired *t*-tests reveal significantly higher glycogen values for the ovary than the testis ($P = >0.05$), except immediately after spawning when

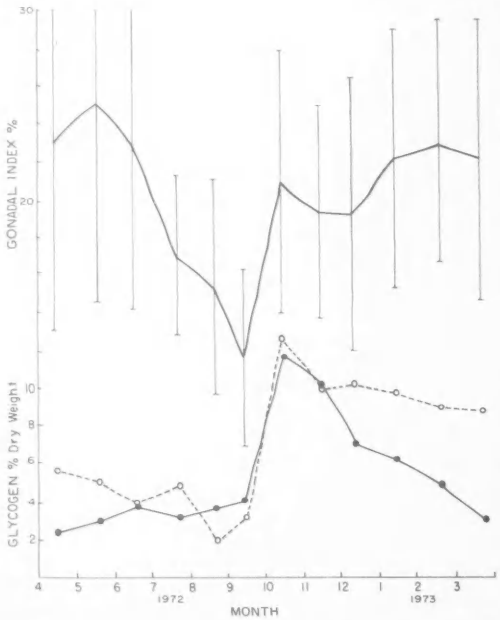

FIG. 1. (*Upper*) Monthly variation of gonadal index (ratio of gonad weight to total wet weight × 100) of the red sea urchin (*Strongylocentrotus franciscanus*) at Amphitrite Point, B.C. (*Lower*) Monthly variation of percentage glycogen in the gonad. Broken line = ovary; solid line = testis.

the ovary is more depleted. The annual mean for females is 7.1% (range 2–13.1%) and for males 5.3% (range 2.4–12.2%).

The gonodal and glycogen indexes are a function of the cytological condition of distinctive cells originating in the acinal germinal layer. Usually called "nutritive phagocytes" (Holland and Giese 1965) these ingest gametes and also function as glycogen repositories, alternating between globulated and deglobulated phases (Caullery 1925). In the former phase these cells are ovoid to subspherical, with a small nucleus, a large vacuole, and numerous conspicuous eosinophilic cytoplasmic globules. As gametogenesis progresses or during inanition the cells lose their cytoplasmic inclusions and eventually appear as nuclei and large vacuoles with no discernible cytoplasm in the germinal epithelium. During the postspawning recovery, the nutritive phagocytes multiply, resume their globulated phase, and ultimately invade the acinal lumen.

There is variability in the gonadal index, glycogen, and histological appearance of individuals from the same population, and these differences are more pronounced in distant populations or those from different environments. However, there is a definite synchronous sequence of gametogenesis (Fig. 2, 3).

The ovarian cycle may be divided into five stages, arbitrarily distinguished by the histology of the developing ova.

Stage 1. Oogonia and primacy oocytes are located at the base of the germinal layer. Early oogonia are present in clusters of packed cells with small, densely staining nuclei. The oogonia divide to form a continuous band of primary oocytes, distinguishable by basophilic cytoplasm and distinct nucleoli.

Stage 2. As the primary oocytes enlarge, their outline becomes irregular, the cytoplasm remains strongly basophilic, and yolk is sparsely present in small globules. The germinal cells are still situated in the acinal wall.

Stage 3. The oocytes migrate towards the acinal lumen and commence a cytoplasmic growth phase. The cytoplasm is only weakly basophilic, the nucleus is large, vitellogenesis is proceeding, and meiotic division is starting.

Stage 4. Reduction division is completed, resulting in ova with small nuclei, scant neutrophilic cytoplasm masked by large quantities of strongly acidophilic yolk. The ova pack the acinal lumina and ovarian ducts.

Stage 5. After spawning the acinal lumina contain numbers of unshed ova undergoing lysis and phagocytosis by the nutritive phagocytes. At this time proliferation of oogonia and primary oocytes is greatest.

All gametic stages except ripe ova are present in varying proportion throughout the year, with ova never exceeding 60%, and spermatozoa 80% of the total cell types enumerated.

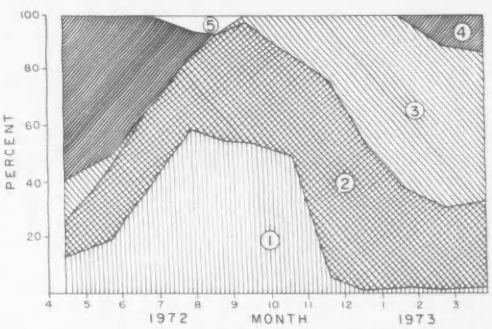

FIG. 2. The oogenetic cycle of the red sea urchin showing percent frequencies of defined stages from April 1972 to March 1973 at Amphitrite Point, B.C.; 1 = oogonia and primary oocytes; 2 = secondary oocytes; 3 = late oocytes; 4 = ova; 5 = relict ova.

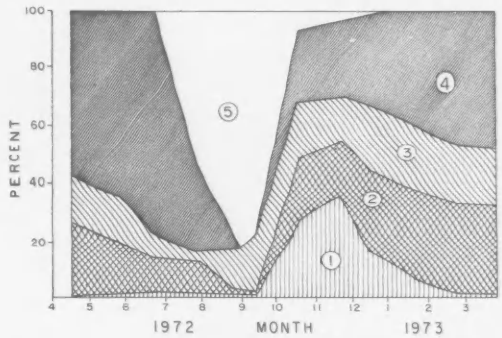

FIG. 3. The spermatogenetic cycle of the red sea urchin showing percent frequencies of defined stages from April 1972 to March 1973 at Amphitrite Point, B.C.; 1 = spermatogonia; 2 = primary spermatocytes; 3 = secondary spermatocytes and spermatids; 4 = spermatozoa; 5 = relict spermatozoa.

The male cycle is similar, but the period of maximum spermatogonia productivity is November to December, some 4 mo later than the female. Mature spermatozoa are present through a larger part of the year and emission is more complete. A chronological division of spermatogenesis is arbitrary, but five stages may be distinguished.

Stage 1. Primary germ cells and young spermatogonia are present in small clusters situated at the base of the germinal layer, each consisting of a small rounded cell with a large nucleus and scant basophilic cytoplasm. Division results in a continuous band of spermatogonia in the acinal wall.

Stage 2. The spermatogonia intergrade with the primary spermatocytes which are characterized by increased size and a nucleus filled with chromatin strands; reduction division gives rise to the secondary spermatocytes.

Stage 3. The secondary spermatocytes migrate to the acinal lumen. Division occurs and produces spherical cells which form the spermatids; these have a small darkly staining nucleus within a thin membrane, but cytoplasm is not discernible.

Stage 4. The spermatids elongate resulting in clusters of spermatozoa occupying most of the acinal lumina.

Stage 5. Numerous spermatozoa are retained after spawning; a few appear to undergo spontaneous lysolysis, but the majority are phagocytosed over a 5-mo period by the nutritive phagocytes. Some relict spermotozoa may be observed within phagocytes situated in the germinal layer.

Mature spermatozoa are present over a longer period than are ova, and spawnings were observed in the field from June to September, with maximal activity in July. By September no new spermatozoa were elaborated, but many relict cells remained. Maximum spermatogonial elaboration occurred in mid-November, and transition to latter stages, including spermatozoa, was uniform until late March, when spermatozoa were produced at the expense of earlier stages.

DISCUSSION

This investigation was concerned with commercial exploitation of red urchins; thus, initial gonad differentiation and variations in gonad yield compared with body size were ignored and only commercially sized individuals (test diam 100+mm) were included in the samples.

It is generally accepted that regular echinoids are exclusively gonochoric. Hermaphrodites rarely occur; the earliest record of them for the genus *Strongylocentrotus* is by Gadd (1907) for *S. droebachiensis*. Boolootian and Moore (1956) described a bisexual red urchin from California that produced normal plutei from self-fertilized gametes. One specimen in the present study was hermaphroditic, a northward extension of a phenomenon thought to occur south of 45°N by Boolootian and Moore (1956).

While latitude may have little influence on the occurrence of hermaphrodites, certain localities have a high frequency of bisexual individuals. For instance, the Palos Verdes area of California has a hermaphrodite *S. purpuratus* ratio of 1:500 (Boolootian and Moore 1959). The presence of even a small proportion of functional hermaphrodites suggests that the gonochoric condition may be labile. This hypothesis may be further supported by the occurrence of populations where the sex ratios depart substantially from equality. In the majority of invertebrates sexuality is genetically determined, but in some cases environmental factors may be determinant. Boolootian (1960) suggested that pronounced temperature variations may influence the sex differentiation of *S. purpuratus*. Though MacBride (1903) detected germinal cells in *Echinus* at metamorphosis, gonad primordia do not appear in the red urchin until the test is 3–5 mm diam and sexual differentiation occurs even later. Populations displaying bias to one sex may be the product of environmental factors acting during the planktonic stages or early after settlement. Gonor (1973c) found that the sex ratio of *S. purpuratus* from Yaquina Head, Oregon, differed significantly from 1:1, and, in another locality, annual monitoring of recruits showed a balanced distribution except for one year, when the proportion of males was 0.61.

This led Gonor to propose that the species was a labile gonochorist, with a multiple, autosomal, sex-determining mechanism that may be influenced by environmental factors. Another explanation for sex-biased samples may be sought in the mechanism and nature of clustering, as a tendency to sexual segregation would fully account for anomalous sex distribution within the sample.

Gonadal index is directly correlated with food availability (Lasker and Giese 1954; Ebert 1968), which influences the condition of the nutritive phagocytes. At Amphitrite Point red urchins show a postspawning minimum gonadal index at the end of September, followed by a rapid recovery due to high cropping during the autumnal disintegration of macroalgae. The increased wet weight of the gonads immediately prior to spawning may be attributable to water uptake, as Miller and Mann (1973) demonstrated a decrease in the dry weight to wet weight ratios for the ripe gonads of *S. droebachiensis*. After water uptake the gonads of the red urchin become fluid and unattractive as food.

Interpretation of the gonadal index is complicated in echinoids because the gonad also functions as a storage organ. Stott (1931) demonstrated the presence of glycogen, and histological observation shows the nutritive phagocytes in the globulated phase to be the sites of glycogen accumulation. The nutritive phagocytes are not motile and Farmanfarmaian and Phillips (1962) showed that they remained within the gonad, and depend upon nutrient transportation from the alimentary system by the perivisceral fluid. When nutrition exceeds metabolic and growth requirements, the phagocytes function analogously to the Leydig cells of molluscs. The glycogen cycle (Fig. 1 *lower*) agrees with the results of Greenfield et al. (1958) for red urchins from California, but the chronology is displaced approximately 2 mo later.

Gonadal growth occurs prior to gametogenesis (Boolootian 1966), and during inadequate assimilation gamete formation proceeds at the expense of reserves; however, in the red urchin, previously stored glycogen is not sufficient for the attainment of ripe condition. During starvation of an individual with normal gonadal index, gametogenesis continues for 3–4 wk, then phagocytosis exceeds gamete production, and the gonad degenerates to a dark brown mass (Bernard unpublished data.) These observations support the hypothesis of Pearse (1969) that substantial stored nutrients are required for the completion of gametogenesis.

There is an inverse correlation between gonadal glycogen and sexual ripeness (Mori et al. 1966). Ovarian glycogen levels are lowest when vitellogenesis is completed, but in the testis, depletion occurs earlier in the reproductive cycle. This sexual difference is the result of glycogen conversion to support the formation of spermatogonia and spermatocytes early in the cycle, and the retention of glycogen by the ovary for conversion to yolk lipids in the final stages of ova production. In both sexes this cycle is analogous to the glucose–fatty acid cycle of vertebrates, and the cost of conversion is small (Krebs 1970).

In the red urchin the nutritive phagocyte and gametogenic cycles are in competition, so that gamete accumulation is not equivalent to production. Throughout the year developing and mature gametes are phagocytized by the nutritive phagocytes; thus, adequate assimilation as well as previously accumulated reserves are a requisite for gametogenesis and successful spawning. The time of optimal proliferation of oogonia and spermatogonia is after the disintegration of relict gametes and the period of maximum food availability. These observations support the hypothesis of Pearse (1969) that seasonal gamete cycles are regulated by mortality of primary oocytes. However, Gonor (1973b) found that absolute numbers of primary oocytes in *S. purpuratus* remain constant through periods of maximum phagocytosis. Data presented here and by Gonor (1973b) suggest that oocytes and spermatocytes convert to gametes in the same year. However, Holland and Giese (1965) reported that oogonia took up isotope labels throughout the year, though the label could not be detected in oocytes and ova when injected early in the session, which leads to the conclusion that oogonia and oocytes are produced in one year and mature the following year.

There is an annual synchronous cycle of gametic maturation in each population, but functional spermatozoa are present over a longer period than ova, so that the female cycle is the determining spawning factor. Spawning is episodic, involving limited groups, even in a continuous population. Chatlynne (1969) and Cochran and Engelmann (1975) considered temperature the major controlling factor. Others identify photoperiod (Boolootian 1966) or food availability (Gonor 1973a, 1973b) among regulators of the reproductive cycle. Latitudinal variation, with spawning occurring progressively later to the north, supports the temperature hypothesis. Potential spawning periods of red urchins occur from February to July at Pacific Grove, California (35°N) (Newmann 1923); in southern British Columbia (49°N) from March to September; and in the Queen Charlotte Islands (52°N) probably from April to October (Bernard unpublished

data). These periods are merely times of potential spawning, determined by the observation of mature gametes in the gonads. There is no evidence that breeding actually extends over such a long period. In the Amphitrite Point population studied, gamete emission was observed June 20 and intermittent episodes of spawning occurred until early September. There appears to be some environmental cue that triggers spawning, as simultaneous emission of gametes occurred in groups of red urchins held in unconnected tanks at the Pacific Biological Station in mid-July. Himmelman (1975) presented evidence that for the green urchin (*S. droebachiensis*) spawning may be cued by the occurrence of the spring phytoplankton bloom. However, the presence of phytoplankters or their metabolites cannot be the sole or universal stimulus, as field-observed spawning frequently involved a group of urchins some tens of metres across, and adjacent individuals were not shedding gametes, though the gonads were equally ripe.

According to Barnes (1975), reproductive synchrony is the result of exogenous regulation, as desynchronizing factors are always present. Even in a stable environment individual variations lead to eventual collapse of synchrony. The situation may be analogous to the model proposed by Lubet (1966) for the bay mussel where the reproductive cycle is controlled internally and synchronized by external factors. In the red urchin the major external factor synchronizing commencement of the cycle is the availability of food during the postspawning period. This period is critical to the glycogen and the gametic cycles and may be endogenously regulated by the same mechanism.

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Lymphocystis Disease in Yellowfin Sole (*Limanda aspera*) in the Bering Sea[1]

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A study to determine the prevalence of fish disease in the Bering Sea revealed lymphocystis disease in yellowfin sole (*Limanda aspera*). The gross pathology and histopathology of typical nodules are described. Electron microscopic studies revealed characteristic icosahedral lymphocystis virus particles, as well as clusters of intracellular structures of uncertain origin. This study begins a larger effort to understand the effects of an altered marine environment upon the biology of lymphocystis virus infection in the Bering Sea.

Key words: lymphocystis, pleuronectids, yellowfin sole, Bering Sea, flatfish.

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La lymphocytose a été mise en évidence chez la limande *Limanda aspera* lors d'une étude des maladies du poisson dans la mer de Béring. L'anatomie macroscopique et pathologique des nodosités typiques est décrite. Le microscope électronique a mis au jour des particules virales icosaèdrales caractéristiques de la maladie, ainsi que des amas de structures intracellulaires d'origine incertaine. Cette étude est un premier jalon dans les tentatives de comprendre les effets d'un environnement marin modifié sur la biologie de la lymphocytose dans la mer de Béring.

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LYMPHOCYSTIS virus is a DNA containing icosahedral virus which has been previously documented to be the etiologic agent responsible for lymphocystis disease in a number of species of marine and freshwater fish. In marine fish the disease has been found in several species of flounders, dabs, and plaice, all members of the teleost family Pleuronectidae, as well as in species belonging to several other teleost families. Lymphocystis has usually been found in the waters of the northern, eastern, and western Atlantic Ocean (Nigrelli and Ruggieri 1965; Murchelano and Bridges, 1976), although there is a report of lymphocystis virus in some tropical waters of the Pacific Ocean, in the Gulf of Panama and the Gulf of California (McCosker and Nigrelli 1971). A survey of fish disease prevalence in the Bering Sea revealed lymphocystis disease in a new host species, the yellowfin sole (*Limanda aspera*), a member of the pleuronectid family. This is the first report of lymphocystis virus in the Bering Sea.

The identification of lymphocystis disease in fish is generally determined by the unique gross and histopathologic appearance of virus-infected cells and the presence of cytoplasmic icosahedral virus particles. The purpose of the present paper is to demonstrate the conformity of the lesions observed in the yellowfin sole with previously published accounts of lymphocystis histopathology and virus ultrastructure. In addition, our studies demonstrate the presence of, as yet, uncharacterized intracytoplasmic fibrillar inclusions that are not clearly of viral origin.

One note of caution that is appropriate in interpreting our findings is that the identification of lympocystis virus identical to the etiologic agent of this disease in other species or geographic localities is presumptive. Because no sensitive technique, such as a radioimmune assay, currently exists that would distinguish closely related but distinct lymphocystis virus types, there may be a

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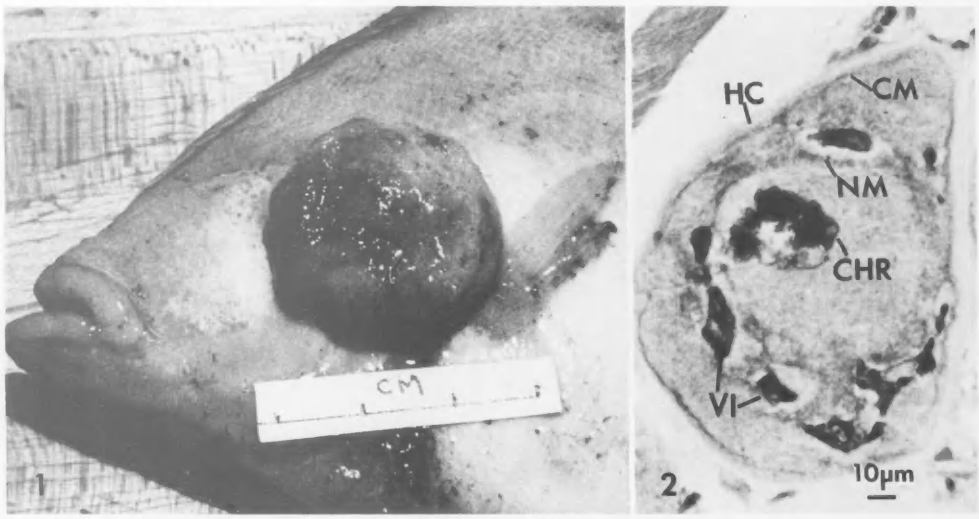


FIGURES 1 AND 2

FIG. 1. Single large lymphocystis nodule at base of pectoral fin on nonpigmented side of yellowfin sole. Individual hypertrophied cells may be seen at arrow. FIG. 2. Lymphocystis infected cell showing hyaline capsule (HC), cell membrane (CM), basophilic cytoplasmic viral inclusions (VI), coarse cytoplasm, and nuclear membrane (NM). The hypertrophied nucleus contains basophilic clumped chromatin (CHR) in a pale eosinophilic background of nuclear matrix. H&E.

spectrum of viruses with the general morphology identified as lymphocystis and capable of causing a generally similar pattern of disease.

Materials and Methods

As part of an environmental assessment program, numerous species of bottom-dwelling fish were collected by bottom trawl. Diseased fish, as well as samples of apparently uninfected controls, were examined for signs of macroscopic parasites, and samples of normal and diseased tissue preserved for later pathologic analysis by light and electron microscopy. Samples preserved for histopathologic observation were fixed in 10% buffered Formalin, later embedded in paraffin, sectioned at 7 μm thickness, mounted on glass slides, and stained with hematoxylin and eosin (H&E). Tissues preserved for electron microscopy were fixed in a modified Karnovsky's solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.06 M phosphate buffer and later embedded in Spurrs low viscocity epoxy resin, thin sectioned at 1 μm thickness, mounted on glass slides, and stained with toluidine blue for light microscopic examination and selection for observations using a Zeiss EM 9S electron microscope.

Results and Discussion

Of 1857 yellowfin sole collected and examined for disease, 79 or 4.3% exhibited the characteristic external surface nodules of lymphocystis disease. No other species collected showed signs of the disease. The gross lesions (Fig. 1) were present as one or more ovoid surface nodules ranging in size from 1 to 25 mm. These nodules were usually white or pale yellow. The hypertrophied infected cells comprising the nodules were sometimes so enlarged as to be visible to the naked eye. The nodules were always on the nonpigmented side of the fish, the side closest to the ocean floor as the fish normally swims. This phenomenon, and the fact that no parasiites that might serve as vectors for the virus were ever regularly observed on infected fish, suggests the virus may be adsorbed onto bottom sediments and transmitted to susceptible fish by physical contact. This has been suggested as a possible route of infection in other epizootics of the disease, although actual mechanisms of transmission are unknown (Russell 1974).

The histopathologic features of the infected cells included cellular hypertrophy, cell enclosure by a distinctive hyaline capsule, enlarged nucleus often in some stage of karyolysis, and granular-appearing cytoplasm with prominent inclusions (Nigrelli and Ruggieri 1965). It is generally accepted that the transformed cell is of fibroblastic origin, although the infected cell undergoes such dramatic morphologic change so as to be un-

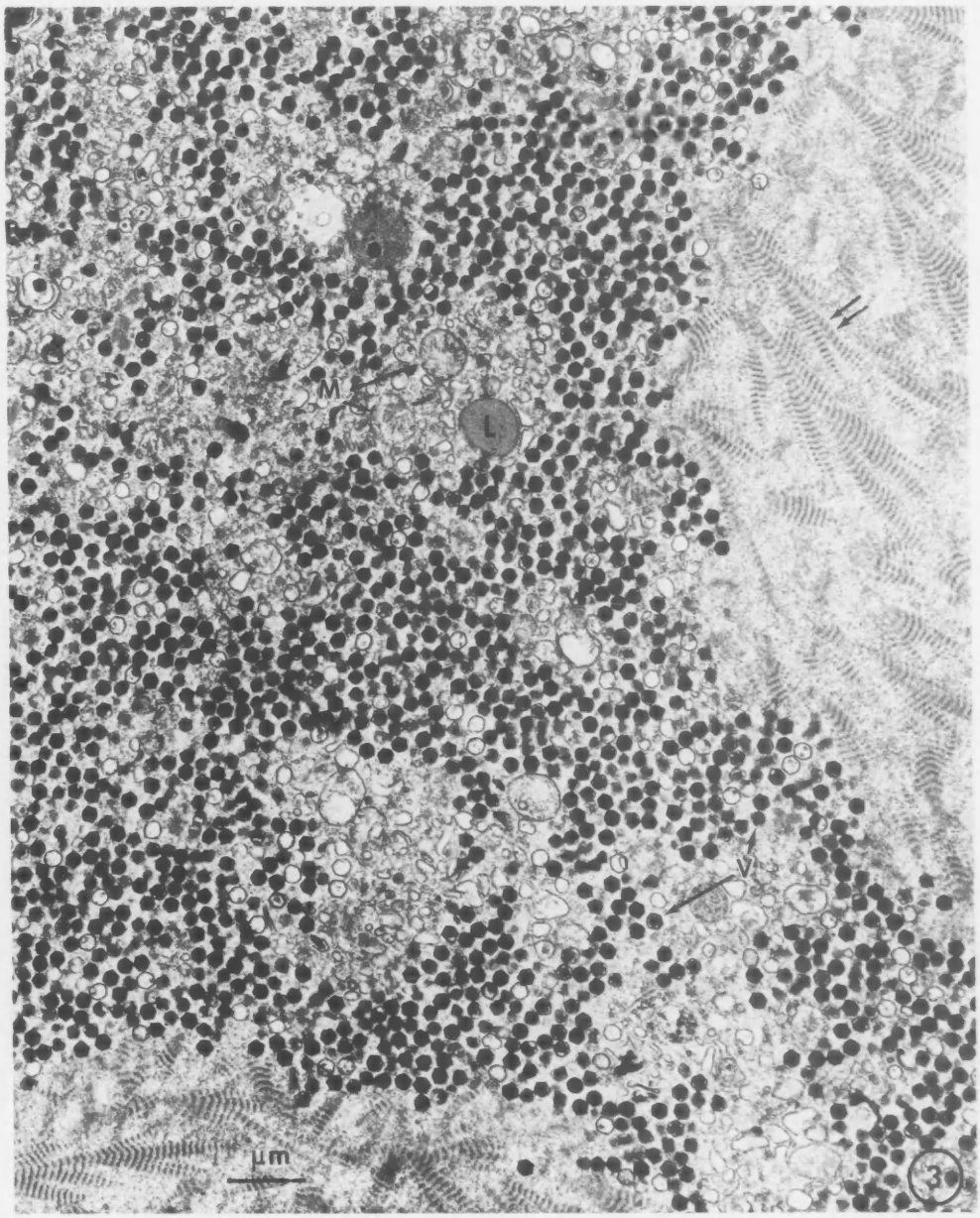
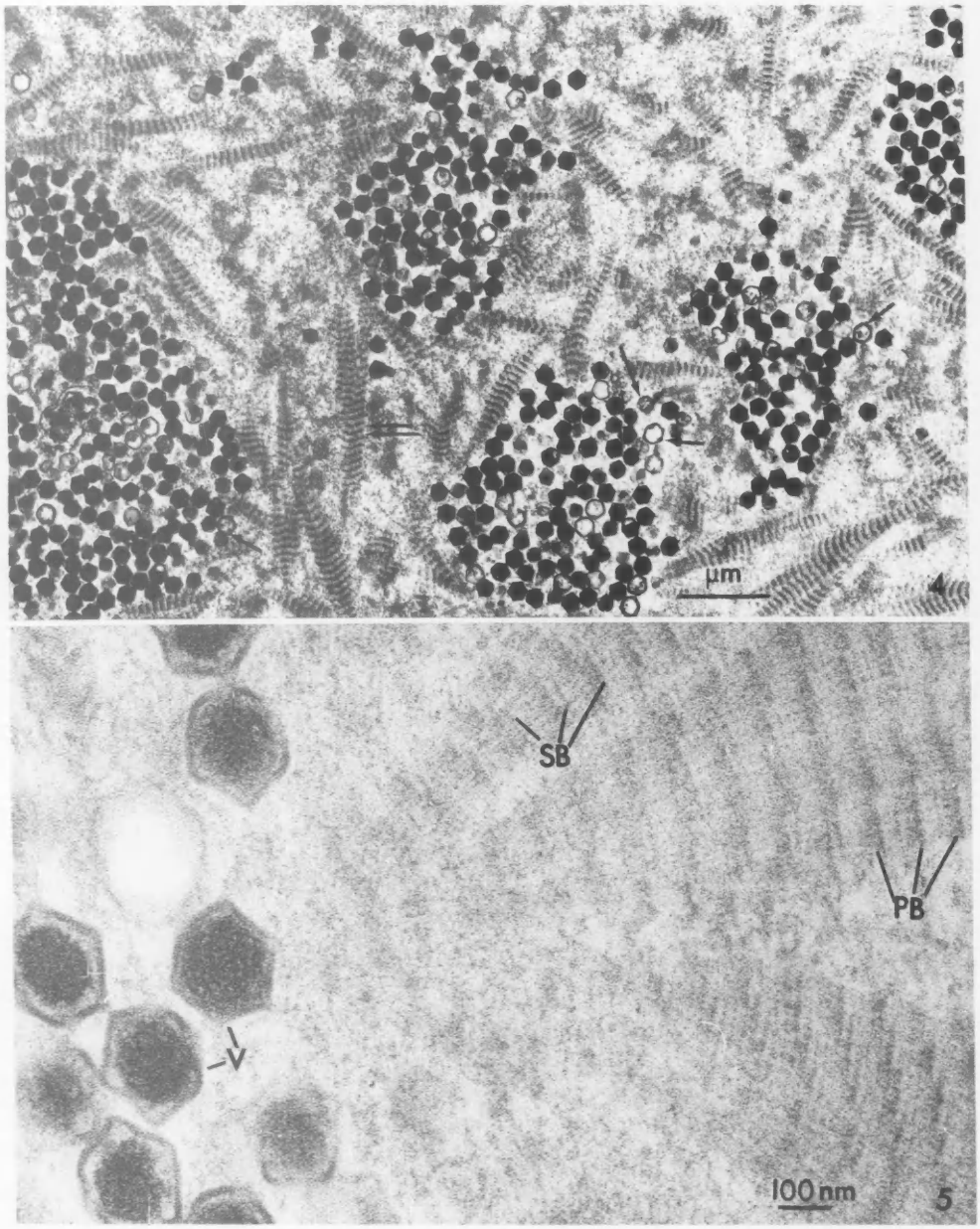


FIG. 3. Electron micrograph of cytoplasm of an infected cell. Note virus particles (V, single arrow), lipid droplet (L), and mitochondria (M). Fibrillar structures with periodic banding (double arrows) are intracytoplasmic and resemble long-spaced collagen.



FIGURES 4 AND 5

FIG. 4. Electron micrograph of cytoplasm of an infected cell containing numerous regular five- and six-sided viral profiles indicative of an icosahedral structure. Some incomplete or empty capsids can also be seen (arrows) as well as the tapered, banded, fibrillar structures (double arrows) observed in Fig. 3. FIG. 5. Higher power view of banded fibrillar structures seen in Fig. 3 and 4. Note presence of small bands (SB) periodically interspaced between more prominent bands (PB). Virus (V) showing dense nucleoids and five- and six-sided capsids can also be seen. The viral particle at bottom left shows electron dense filaments extending from the nucleoid to the viral capsids.

recognizable as originating from a specific cell type (Weissenberg 1965). The microscopic appearance of our samples was uniformly consistent with these features of lymphocystis disease (Fig. 2). Within affected tissues we observed fibrosis, epithelial hyperplasia, blood vessel proliferation, engorgement of vessels with some extravasation of red blood cells and macrophages, all features associated with inflammatory processes in pleuronectids.

Other studies of the disease have focused on identification and characterization of the lymphocystis virus. The virus particles range in size from 130 to 300 nm (Russell 1974). They may often be seen in crystalline array and are always located in the cytoplasm, in conjunction with the inclusion bodies seen on light microscopy (Walker and Weissenberg 1965). Although studies of cultured fish cell lines confirm that intracellular viral aggregates occur by processes of viral replication and assembly and not simply by infection and accumulation, there is no evidence of viral budding or lysis of infected cells (Midlige and Malsberger 1968). Rather, infected cells seem to hypertrophy indefinitely as a result of viral infection.

A number of viral forms approximately 180–190 nm can be seen by use of electron microscopy in a portion of the cytoplasm of an infected cell (Fig. 3, 4). These include regular five- and six-sided particles consistent with an icosahedral structure, a number of empty capsids, and numerous cartwheel-like particles that have electron-dense filaments extending from the nucleoids to the viral capsids (Fig. 5).

Our ultrastructural studies of the lymphocystis infected cells reveal that the clumps of virus in the cytoplasm are associated with alterations of normal intracellular organization. In particular, we observe numerous, randomly arranged fibrillar forms throughout the cytoplasm of infected cells. The structures indicated by double arrows in Fig. 3 and 4 suggest on initial inspection that they may be collagen, fibrin, some form of myotonic structure, or possibly a site for virus replication and assembly. Analysis at higher powers reveals the presence of regular bands in these structures at approximately 90-nm intervals (Fig. 4). The value usually given for intervals between the regular bands of collagen is 64 nm, but the value of 90 nm is consistent with so-called long-spaced collagen. The tapered appearance of these structures, however, and their intracellular location are not usually associated with collagen. The random arrangement of the fibrillar structures and the presence of intermediate smaller bands seen at higher powers are similarly not consistent with smooth muscle elements or other types of known contractile apparatus. Further, we know of no known precedent for large clumps of intracellular fibrin. Although we do not feel it is possible to conclusively identify these structures at this time, we suggest that since the structures are not found in normal, uninfected fish fibroblasts, they might be related to the process of viral infection. Previous ultrastructural studies of lymphocystis infections by Walker and Weissenberg (1965), Zwillenberg and Wolf (1968), and Midlige and Malsberger (1968) have not reported similar intracytoplasmic inclusions. The other most reasonable explanation is that the fibrillar structures are long-spaced collagen, but this hypothesis does not explain their intracellular location.

Studies of the epidemiology of lymphocystis disease in the Bering Sea are currently being prepared. Because the ecology of the Bering Sea may undergo some dramatic change in the near future, owing to the probable introduction of offshore oil explorations, the presence of lymphocystis disease is of more than passing interest. Changes in marine environmental conditions, such as water salinity, temperature, and the amount of sewage and pollutants, have been repeatedly associated with other forms of proliferating growths in fish (Young 1964; Wellings 1969; Cooper and Keller 1969; Dawe and Harshbarger 1975). In at least one of these, the epidermal papillomas of pleuronectids, a viral etiology is also suspected. Comparative pathologists are seeking to define the direct and indirect relationships between specific changes in the environment and possible resultant patterns of disease expression and pathogenesis. The possible permissive effects of various ecological disruptions in facilitating lymphocystis disease is a question which will be worthwhile to pursue.

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A Biochemical Genetic Study of Zoogeography of Lake Whitefish (*Coregonus clupeaformis*) in Western Canada[1]

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FRANZIN, W. G., AND J. W. CLAYTON. 1977. A biochemical genetic study of zoogeography of lake whitefish (*Coregonus clupeaformis*) in western Canada. J. Fish. Res. Board Can. 34: 617–625.

Frequencies of alleles of the genes governing electrophoretic phenotypes of lake whitefish (*Coregonus clupeaformis*) muscle glycerol-3-phosphate dehydrogenase (G-3-PDH) and lactate dehydrogenase (LDH) enzymes, the genetics of which were determined previously, proved useful tools for characterizing populations. Malate dehydrogenase (MDH) and hemoglobin electrophoretic phenotypes, the genetics of which have not been determined, proved useful only for discerning differences among large groups of populations. Using all these characters we determined postglacial routes of gene flow among western Canadian lake whitefish populations and related the biochemical data to the glacial refugia in which lake whitefish are believed to have survived the Wisconsin glaciation. Apparently most lake whitefish populations west of the Ontario–Manitoba boundary and east of the Rocky Mountains were derived from a Mississippi refugium stock with some input from the Bering refugium. At an early stage in the mixing of the two stocks, headwaters of the Peace, Athabasca, and Mackenzie rivers and the Fraser River system were invaded. Subsequently, these areas were cut off from mainstem rivers of the plains, and a G-3-PDH allele not found in early emigrants from the Mississippi refugium appeared and spread throughout the major river systems of central Canada. No evidence was found that any Mississippi stock lake whitefish were able to invade the upper Liard River and Yukon River system including the area of the Bering refugium. Bering stock lake whitefish probably emigrated from that refugium by way of headwater transfer from the Yukon River to the Liard River and possibly, by way of the Porcupine and Peel rivers, from the Yukon River to the lower Mackenzie River. Routes of postglacial dispersal suggested by geological information, rather than selection, appear to provide adequate explanations for the distributions of the biochemical characters in present day lake whitefish populations. This explanation of the biochemical data is also in accord with the distribution of modal gillraker counts in lake whitefish populations and also with the distribution of other freshwater fishes in western Canada.

Key words: fish, lake whitefish, *Coregonus clupeaformis*, salmonidae, zoogeography, dehydrogenase, postglacial, biochemical genetics

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La fréquence des allèles responsables des différences de phénotypes observées lors de l'électrophorèse des enzymes musculaires glycérol-3 phosphate-déshydrogénase (G-3-PDH) et lactate-déshydrogénase (LDH) du corégone de lac (*Coregonus clupeaformis*), dont la composition génétique avait été déterminée antérieurement s'est montrée utile à la caractérisation des populations. Les phénotypes électrophorétiques du malate-déshydrogénase (MDH) et l'hémoglobine, dont la composition génétique n'a pas été démontrée, n'ont servi qu'à discerner les différences entre les groupes nombreux. À partir de toutes ces caractéristiques, nous avons déterminé les voies post-glaciaires de la dispersion des gènes parmi les populations de corégones de lac de l'Ouest canadien et établi une relation entre les données biochimiques et les refuges glaciaires où les corégones auraient survécu à la glaciation du Wisconsin. Il semblerait que la plupart des populations à l'ouest de la frontière entre l'Ontario et le Manitoba et à l'est des montagnes Rocheuses proviennent du refuge du Mississipi et dans une moindre mesure de celui

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de Béring. Au début du mélange des deux stocks, les eaux de tête des rivières de la Paix et Athabaska et du fleuve Mackenzie, ainsi que la bassin du fleuve Fraser ont été envahis. Par la suite, ces régions ont été coupées des principaux cours d'eau des plaines, et un allèle du G-3-PDH, absent chez les premiers émigrants du refuge du Mississipi est apparu et s'est répandu chez les poissons des principaux bassins hydrographiques du centre du Canada. On n'a rien trouvé qui indiquait que des corégones de lac du stock du Mississipi aient pu envahir le cours supérieur de la rivière Liard et le bassin du fleuve Yukon, y compris la région du refuge de Béring. Les corégones de lac de ce dernier refuge ont probablement émigré par la voie d'un transfert de la tête du fleuve Yukon à la rivière Liard et, aussi par la voie des rivières Porcupine et Peel, du fleuve Yukon au bas-Mackenzie. Les voies de dispersion post-glaciaire indiquées par les données géologiques, plutôt que par la sélection, semblent expliquer de façon satisfaisante la distribution des caractères biochimiques dans les populations actuelles de corégones de lac. Cette explication des données biochimiques est également en accord avec la distribution des fréquences maximales de branchicténies dans les populations de corégones de lac et avec la distribution d'autres espèces dulcicoles de l'Ouest du Canada.

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NORTH American lake whitefish (*Coregonus clupeaformis*) have been variously described as several species or as forms of a single species. Lindsey et al. (1970) described lake whitefish as a "species complex", based on observations across North America of modal gillraker counts, the existence of more than one morphological form of lake whitefish in some lakes, and some preliminary observations of variation in electrophoretic phenotypes of three proteins, lactate dehydrogenase (LDH), glycerol-3-phosphate dehydrogenase (G-3-PDH) and hemoglobin. The genetic bases of the LDH and G-3-PDH polymorphisms now have been determined (Clayton and Franzin 1970; Clayton et al. 1973). This paper describes the distribution in western Canada of population gene frequencies of lake whitefish LDH and G-3-PDH alleles as revealed by their electrophoretic phenotypes.

Lake whitefish in western Canada carry two alleles at the locus coding for the most anodal of two forms of heart-type LDH subunits (Clayton and Franzin 1970). Originally, we designated these alleles h^b and h^c. Recently, a very satisfactory and comprehensive system of nomenclature for LDH genes in salmonids has been proposed (Bailey et al. 1976). In this new system the alleles formerly designated h^b and h^c become lake whitefish $ldhH\alpha^A$ and $ldhH\alpha^B$, respectively. Lake whitefish populations in western Canada are described simply by the frequency of the $ldhH\alpha^A$ allele.

Clayton et al. (1973) described a genetic and molecular model to explain observed electrophoretic phenotypes of G-3-PDH from lake whitefish white muscle. The model proposed the existence of two nondominant alleles, a^1 and a^2 coding for A-type subunits and three nondominant alleles, b^1, b^2, and b^3 coding for B-type subunits. Lake whitefish populations in this paper are described by the frequency of the a^1 allele and by frequencies of all three b alleles.

Many phenotypes for lake whitefish hemoglobin are revealed by starch-gel electrophoresis (Franzin, 1974) but the genetic relationships among them remain unclear at present. Despite the impressive diversity of phenotypes some important simplifications can be made (Franzin 1974) and the distribution of the phenotypes among lake whitefish populations is of some utility in the present study.

The use of MDH phenotypes is similarly limited by an incomplete understanding of the underlying genetic system. Although there is variation between individual lake whitefish in the electrophoretic phenotype for supernatant MDH (Franzin 1974) that is very reminiscent of the phenotypic variability found in Pacific salmon and rainbow trout (Bailey et al. 1970, Numachi et al. 1972, Clayton et al. 1975), the genetic relationships among the lake whitefish MDH phenotypes are obscured by factors that control the intensity of MDH isozymes in lake whitefish tissues. These unknown factors that seem to affect the intensity of staining of MDH isozymes in lake whitefish are evidently not operative in rainbow trout or chinook salmon. In parallel with the hemoglobin phenotypes, the distribution of supernatant MDH phenotypes in lake whitefish populations is of some interest in this study even though the genetics of the polymorphism remains obscure.

In this report the distributions of some alleles for LDH and G-3-PDH as well as some phenotypes of MDH and hemoglobin in lake whitefish populations are explained by a scheme of post-Wisconsin dispersal of lake whitefish from glacial refugia. Two major glacial refugia will be considered: the Bering refugium in central Alaska and western Yukon and the Mississippi–Missouri refugium south of the major ice sheets in the

northern plains of the United States. We will assume that identical electrophoretic phenotypes are the products of identical genotypes and that the organism can be treated as a single biological species as defined by Mayr (1964). Also, although the history of ice advance and retreat is of utmost importance relative to zoogeography of lake whitefish (or for that matter most northern North American freshwater fishes), the events of Wisconsin glaciation and deglaciation will not be discussed at length here. Background information of varying scope and detail regarding events of the Wisconsin glacial period may be found in Lindsey et al. (1970), McPhail and Lindsey (1970), and Prest (1970).

Materials and Methods

Field Collections

Lake whitefish for biochemical analyses were collected mostly by gillnet during the period 1967–72 from 38 locations in western Canada. Transport and storage of fish and sampling methods for blood and muscle tissue are described by Lindsey et al. (1970) and Clayton and Franzin (1970).

Biochemical Analyses

Lactate dehydrogenase phenotypes were visualized using methods described by Clayton and Franzin (1970). Glycerol-3-phosphate dehydrogenase phenotypes were determined by the method of Clayton et al. (1973). Analyses for malate dehydrogenase activity on starch gels were carried out as described for walleye by Clayton et al. (1971) except for the following changes which were made to clarify the lake whitefish electrophoretic phenotypes:

1) Tissue samples were macerated in a 1:3 ratio by weight of tissue, to a 300 mg/ℓ solution of nicotinamide adenine dinucleotide (NAD).

2) The electrophoresis buffer was *N*-(3-aminopropyl) morpholine-citrate, pH 6.1 at 22°C as described by Clayton and Tretiak (1972). NAD was added to gel and bridge buffers to a concentration of 100 mg/ℓ.

Hemoglobin analyses were carried out as described by Lindsey et al. (1970). In total, 2200 lake whitefish were analyzed for G-3-PDH phenotypes, 1384 for LDH phenotypes, and 768 for hemoglobin phenotypes.

Results

The geographic distribution of the *ldhH*α alleles (Fig. 1A) shows that complete homozygosity for the *ldhH*α^B allele in populations in the Yukon River system and the upper Liard River sets off these populations from those of the rest of western Canada. Outside of these two areas the frequency of the allele is rather uniformly low except for two populations, No. 17 in Lac la Hache and No. 35 in Steeprock Lake, both of which are in headwater lakes.

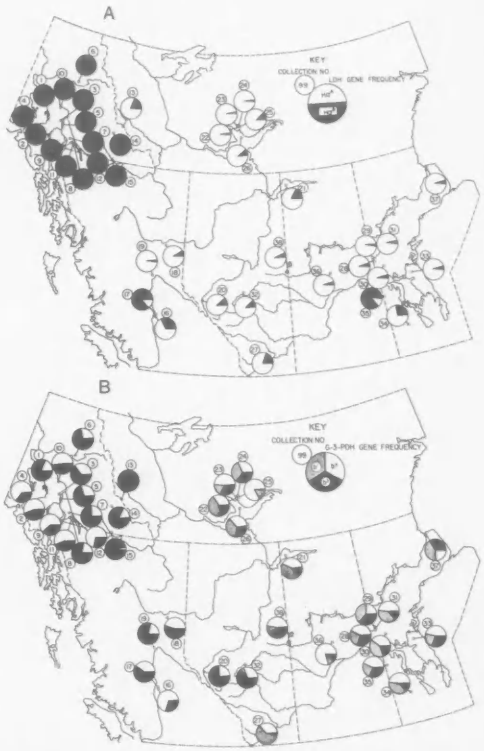

FIG. 1. Gene frequencies in lake whitefish (*Coregonus clupeaformis*); A, lactate dehydrogenase, *ldhH*α alleles; B, L-glycerol-3-phosphate dehydrogenase, G-3-PDH *b* alleles.

The geographic distribution of the three alleles b^1, b^2, and b^3 of G-3-PDH (Fig. 1B) also shows that populations in the Yukon River system and upper Liard River are set off from those of the central plains. In this instance, the Yukon River and upper Liard River populations lack the G-3-PDH b^1 allele. The absence of this allele is shared by a number of other populations all in headwater lakes. These populations include No. 13 in Cache Lake on the upper Nahanni River, No. 16 and 17 in Williams Lake and Lac la Hache, respectively, on the Fraser River system, No. 18 in Moberly Lake on the upper Peace River, and No. 20, in Talbot Lake, an Athabasca River headwater. The distributions of b^2 and b^3 alleles alone without regard to b^1 are rather variable within and outside of the Yukon River sys-

TABLE 1. Distribution of phenotypes for hemoglobin and supernatant malate dehydrogenase in lake whitefish (*Coregonus clupeaformis*) populations. +, means present; −, not found.

Watershed	Hemoglobin			Malate dehydrogenase				
	SS	SF	FF	S_4	S_3F	S_2F_2	SF_3	F_4
Yukon and Alsek rivers	+	+	−	−	−	−	−	+
Liard River (above canyon)	+	+	−	−	−	−	−	+
Fraser River	−	+	+	+	+	+	+	+
Mackenzie River	+	+	+	+	+	+	+	+
Saskatchewan–Nelson River system	+	+	+	+	+	+	+	+
Churchill River	+	+	+	+	+	+	+	+

TABLE 2. Simple correlation coefficients: lake whitefish field collections. MJT = mean July air temperature (degrees Celsius).

Variable		1	2	3	4	5	6	7	8
A. All samples. Significance at 5%* = 0.320, 1%** = 0.413; $N = 38$, df = 36.									
Lat.	1	1.000							
Long.	2	0.563**	1.000						
Elevation	3	−0.106	0.527**	1.000					
MJT	4	−0.559**	−0.639**	0.092	1.000				
G-3-PDH a^1	5	0.307	0.382*	0.124	−0.220	1.000			
G-3-PDH b^1	6	0.300	−0.789**	−0.477**	0.393*	−0.295	1.000		
G-3-PDH b^2	7	0.298	0.618**	0.335*	−0.337*	0.072	−0.723**	1.000	
ldhHα^A	8	−0.431**	−0.780**	−0.356*	0.461**	−0.464**	0.638**	−0.487**	1.000
B. Yukon River samples (including Alsek River). Significance at 5%* = 0.576, 1%** = 0.708; $N = 12$, df = 10.									
Lat.	1	1.000							
Long.	2	0.378	1.000						
Elevation	3	−0.415	−0.060	1.000					
MJT	4	0.423	−0.643*	−0.332	1.000				
G-3-PDH a^1	5	−0.055	−0.580*	0.042	0.504	1.000			
G-3-PDH b^1	6	0.000	0.000	0.000	0.000	0.000	0.000		
G-3-PDH b^2	7	0.279	−0.004	0.099	0.202	−0.320	0.000	1.000	
ldhHα^A	8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C. Non-Yukon River samples. Significance at 5%* = 0.388, 1%** = 0.496; $N = 26$, df = 24.									
Lat.	1	1.000							
Long.	2	0.291	1.000						
Elevation	3	−0.300	0.527**	1.000					
MJT	4	−0.394*	−0.348	0.347	1.000				
G-3-PDH a^1	5	0.100	−0.305	−0.218	0.296	1.000			
G-3-PDH b^1	6	−0.010	−0.684**	−0.411*	0.093	0.188	1.000		
G-3-PDH b^2	7	0.154	0.660**	0.293	−0.233	−0.164	−0.747**	1.000	
ldhHα^A	8	0.003	−0.409*	−0.251	−0.073	0.074	0.382	−0.440*	1.000

tem, but b^2 tends to be in higher frequency in western populations.

The distributions of G-3-PDH alleles a^1 and a^2 are not amenable to the type of presentation in Fig. 1 because of the generally very low frequencies of a^1. However, these data appear in Appendix 1, along with actual figures for the other gene frequencies, and indicate a sort of "epicenter" of highest a^1 frequencies in central Yukon Territory with lesser frequencies elsewhere.

A qualitative distribution of phenotypes for hemoglobin and MDH (Table 1) demonstrates the same discontinuity in lake whitefish populations seen previously in distributions of LDH and G-3-PDH gene frequencies. Populations of lake whitefish in the Yukon River watershed and the upper Liard River watershed are unique and distinct in that they lack the FF hemoglobin phenotype and have exclusively the F_4 supernatant MDH phenotype, while most populations from other waters carry the full range of phenotypes. The designation of phenotypes for hemoglobin as SS, SF, FF is meant to imply slow or fast electrophoretic mobility. Likewise, the S_4, S_3F etc. designation for MDH phenotypes represents the five phenotypes that appear to be derived from combinations of electrophoretically slow and fast MDH subunits. Although salmonid MDH occurs in a dimeric form like other supernatant MDH's it is necessary to use a designation that provides for four doses of MDH subunit in the phenotype because the salmonid supernatant MDH genes are known to have been reduplicated (Bailey et al. 1970, Numachi et al. 1972, Clayton et al. 1975).

Part A of Table 2 shows simple correlation coefficients for gene frequency data with a number of geographic and climatic factors as well as for

among the genes themselves. Parts B and C of Table 2 treat separately the data from Yukon and non-Yukon populations. The separation was based on the apparent geological barrier to lake whitefish movement and hence gene flow into Yukon watersheds from without, and also on the absence of G-3-PDH b^1 and $ldhH_{\alpha}{}^A$ alleles from Yukon collections. This break is also evident in the distribution of hemoglobin and MDH phenotypes (Table 1). Most of the correlation found when all samples were taken together was removed by the separation of the samples into the Yukon and non-Yukon groups. Within the Yukon group, the only significant correlation is between G-3-PDH a^1 frequency and longitude (Table 2, B). On the other hand there are six significant correlation coefficients (Table 2, C) among the variables for the non-Yukon group of samples. Among these variables, only the negative correlations of G-3-PDH b^1 and $ldhH_{\alpha}{}^A$ and the positive correlation of G-3-PDH b^2, all with longitude, seem to be important.

Discussion

In the context of geological history, there are two possible explanations for the observed distributions of lake whitefish population gene frequencies across western Canada. First, one could ascribe the observed distributions to a postglacial history of mutation and selection continuing to the present. According to this hypothesis one would expect to observe the result of selection as clines in gene frequencies in response to climatic or other environmental gradients over the considerable geographic range of the species. Alternatively, the observed genetic variation could be the result of postglacial admixture of two or more slightly different genetic stocks of lake whitefish, subsequent to a period of mutation and possibly differential selection in the two or more widely separated glacial refugia. According to this hypothesis one would expect to see in the present array of population gene frequencies effects of routes of and barriers to postglacial dispersal.

The latter hypothesis seems to provide the most satisfactory explanation of the available evidence. The fact that the G-3-PDH b^1 allele is absent from Yukon watersheds, the Fraser River watershed, and the western headwaters of the northern and central rivers (Mackenzie, Athabasca, and Peace rivers) suggests that the occurrence of this allele has been governed by the postglacial dispersal of lake whitefish from a Mississippi refugium. A similar scheme fits the occurrence of the $ldhH_{\alpha}{}^A$ allele, although its more widespread occurrence (i.e. into Fraser River watersheds and northern plains river headwaters) suggests that perhaps lake whitefish colonization of the western part of central Canada came in two phases, an early wave in which $ldhH_{\alpha}{}^A$ was carried and G-3-PDH b^1 was not and a later wave in which the latter allele was also carried.

The G-3-PDH b^2 allele is ubiquitous[2] in all populations sampled and population gene frequencies of this allele show positive correlation with longitude (Table 2, A,–C). There are three possible explanations for this observation: (1) lake whitefish in both refugia possessed the b^2 allele, but the development of the b^1 allele in the Mississippi refugium early in postglacial time led to a partial displacement of the b^2 allele in early southern populations, through selective advantage of the b^1 allele; (2) lake whitefish in both refugia possessed the b^2 allele, but the greater contribution of Bering refugium emigrants carrying only b^2 and b^3 G-3-PDH alleles raised the population frequencies of the b^2 in western central Canadian lake whitefish populations; and (3) the G-3-PDH b^2 allele had its origin in the Bering refugium and its presence as far east as Hudson Bay is the result of postglacial dispersal. The last hypothesis is supported by the presence of $ldhH_{\alpha}{}^B$ allele and G-3-PDH a^1 allele equally far eastward, both of apparent Bering origin. That the Bering form of *Prosopium cylindraceum*, the round whitefish, has spread all the way to Hudson Bay (McPhail and Lindsey 1970) adds credence to this hypothesis. G-3-PDH b^2 allele is absent from some eastern Canadian lake whitefish populations (unpublished data); this also supports a Bering origin for the b^2 allele, although the far eastern lake whitefish populations may have been derived from an Atlantic refugium. The evidence at hand does not permit a conclusive choice from among these three hypotheses, but support is greatest for the last one.

Support for a postglacial selection hypothesis would be provided by correlation between population gene frequencies and climatic or environmental gradients. This hypothesis may be examined by means of the correlation coefficients among the gene frequency data and a number of geographic and climatic factors (Table 2, A–C).

Most of the correlation found when all samples were taken together (Table 2, A) was removed by the separation of the samples into the

[2]G-3-PDH b^3 allele is also ubiquitous, but calculations using frequencies of this allele provide no further information once b^2 and b^4 frequencies have been used to determine correlation coefficients. Frequencies of b^2 were chosen over b^4 because b^2 frequencies were generally the higher of these two G-3-PDH alleles found in Yukon populations.

Yukon (Table 2, B) and non-Yukon (Table 2, C) groups. A logical conclusion is that much of the correlation found initially was the result of a mixture of two at least partially discrete groups of populations. The only significant correlation of biochemical data with physical or geographical data found in Table 2, B, is a negative correlation of G-3-PDH a^1 with longitude. This result derives from very limited data from a few headwater lakes (Appendix 1). While selection cannot be ruled out, neither can the possibility that founder effect and/or genetic drift have produced high frequencies of G-3-PDH a^1 in the lake whitefish populations of a few Yukon lakes.

In lake whitefish populations outside the Yukon (Table 2, C) the negative correlations of G-3-PDH b^1 and $ldhH\alpha^A$ with longitude may be equally well explained on the basis of fish dispersal as by any selection hypothesis. Thus, it is possible that these alleles have, in locations of high longitude near the Yukon, been diluted by other alleles carried into the area by migrants from the Bering refugium. The strong positive correlation of G-3-PDH b^2 with longitude would appear to confirm this migration hypothesis. Likewise, the negative correlation of $ldhH\alpha^A$ with G-3-PDH b^2 may also be accounted for by the mixing of two stocks, one that carried only the $ldhH\alpha^A$ allele in combination ultimately with all three G-3-PDH alleles (Mississippi refugium stock) with another stock that carried only the $ldhH\alpha^B$ allele together with G-3-PDH b^2 and b^3 but with the b^2 allele more abundant than b^3 (Bering refugium stock).

Clearly, observed correlation, negative and positive, between population gene frequencies and climatic and geographic factors is more consistent with postglacial dispersal movements than with postglacial selection. Further support for this conclusion was indicated previously by data from hemoglobin and MDH phenotype distributions. In no case was correlation observed that was inconsistent with expectations based on knowledge of glaciation and deglaciation.

Postglacial Dispersal of Lake Whitefish

Observed variation in lake whitefish population gene frequencies together with glacial history suggest the following: (1) lake whitefish from southern origins were not able to invade Yukon River or upper Liard River (above Liard Canyon) watersheds; (2) lake whitefish from Yukon River watersheds emigrated from the area of the Bering refugium, probably via headwater connections between the Yukon and Liard rivers; (3) lake whitefish that emigrated from the Mississippi refugium apparently came in two phases, the first during a time of high water levels along the east slope of the Rocky Mountains at which time watersheds of Peace–Mackenzie and Fraser rivers were connected; and (4) Lake whitefish that entered upper Peace River (above Peace Canyon) and the Fraser River system did so after an initial mixing of Bering and Mississippi stocks, but prior to a second phase of Mississippi emigrants.

Support for these statements is supplied by the distributions of many species and morphological forms of species of freshwater fishes as described by McPhail and Lindsey (1970). Only one species, *Prosopium williamsoni*, mountain whitefish, and one form of *Salvelinus malma*, Dolly Varden, both from the Pacific refugium have invaded the upper Liard River. *Prosopium williamsoni* must have entered by way of an inland freshwater route since it is intolerant of sea water. This generally riverine species is found also in the Peace–Athabasca system and conceivably could have been the one species to ascend Liard Canyon. *Salvelinus malma* is capable of entering the sea, thus having two possible routes, inland or via the sea and the Yukon River, eventually reaching the upper Liard. For both species, populations exist in areas where upper Liard River tributaries and Pacific watersheds are closely juxtaposed; local glacial action could have accounted for direct transfer between the different drainages. Only two species have entered the Yukon River system directly, presumably by a fairly recent invasion via the Peel River, a tributary of the lower Mackenzie, and the Porcupine River, a Yukon headwater river (McPhail and Lindsey 1970). Recent data (Bodaly and Lindsey 1977) on LDH and G-3-PDH gene frequencies for lake whitefish populations in one lake tributary to the Peel River and two lakes tributary to the Porcupine River indicate that lake whitefish from these areas are of Yukon River type (i.e. of Bering stock). Apparently, lake whitefish may have emigrated from the Yukon River system in this area but probably have not entered the Yukon River system from the Mackenzie River (or Mississippi refugium). Similarily, lake whitefish taken from a lake tributary to the Liard River immediately below the Liard Canyon are of Yukon River type (R.A. Bodaly, University of Manitoba, Winnipeg, personal communication) indicating that lake whitefish almost certainly emigrated from the Bering refugium by way of headwater transfer from the Yukon River to the Liard River and thence into the waters of central Canada. In summary, information from fish distributions con-

firms hypotheses on lake whitefish dispersal based on the distribution of gene frequencies of biochemical characters.

Chronology of Lake Whitefish Dispersal

With the biological and geological information at hand, it is possible to present a tentative chronology for the postglacial dispersal and distribution of lake whitefish in western Canada. Although some geological dates will be suggested, most dates which follow may be regarded in a relative sense only.

There are probably three main stages in this chronology, namely: (1) glacial, (2) early deglaciation, and (3) postglacial to recent. During the glacial period, until about 14,000–16,000 B.P. (= years before present), lake whitefish survived in the Bering and Mississippi refugia. We might envisage their genotypes, in terms of the proteins for which the genetics have been determined, and the sequence of events as follows: (1) Glacial period: (a) Bering refugium $ldhH_{\alpha}{}^{B}$ allele only, G-3-PDH b^2 and b^3 alleles only, G-3-PDH-a^1 and a^2 alleles present; (b) Mississippi (and Missouri) refugium, $ldhH_{\alpha}{}^{A}$ allele only. G-3-PDH b^3 and a^2 alleles plus possibly b^2 allele. (2) Early deglaciation: 10,000–12,000 B.P. Fish spread out of refugia, intermixing in central Canada. Lake whitefish carrying $ldhH_{\alpha}{}^{A}$ and $ldhH_{\alpha}{}^{B}$, G-3-PDH a^2, b^2, and b^3 entered upper Peace River and Fraser River watersheds via glacial lakes Peace and Prince George, and also headwaters of Athabasca and Mackenzie rivers. As deglaciation continued water levels in Lake Peace lowered, fish could no longer ascend Peace Canyon or easily enter headwaters of western rivers. Movement of fishes down Mackenzie River began and lake whitefish carrying a^1 allele of G-3-PDH began "leaking" out of Yukon via Liard River. The G-3-PDH b^1 allele appeared among fish from the Mississippi refugium, and this allele spread through the central and northern plains areas via glacial lakes Agassiz, Tyrrell, and/or McConnell. The G-3-PDH a^1 allele spread eastward. The G-3-PDH b^1 allele may have developed in the east (where it is in high frequency in some present populations) and spread to central Canada via the Great Lakes–Lake Agassiz connections. (3) Postglacial to recent: the lake whitefish distribution was becoming discontinuous; headwater populations were becoming isolated with concomitant genetic drift.

In this manner we have arrived at the distribution of lake whitefish population gene frequencies that we see today. The break between lake whitefish populations in the Yukon and central Canada and British Columbia is probably a result of the mixture of a large portion of Mississippi stock with a small portion of Bering stock. Subsequent to the initial mixing of the two stocks, lake whitefish may have entered upper Peace and Fraser River watersheds as well as several other western headwaters. These populations were then isolated. In central Canada the mixing of the two stocks probably has continued to some degree to present times by means of unidirectional gene flow out of the Bering refugium.

Selection

During long isolation in the two widely separated refugia, lake whitefish must have been subjected to different sets of conditions of day length, growing season, winter ice cover, mean annual temperature etc., all factors favorable to the development of differential selection in the two areas. Selection and mutation in two geographically well-separated refugia probably led to the development of two separate stocks of lake whitefish, both in terms of biochemical characters, such as those used in the present study, and also in terms of morphometric characters, such as gillraker number. In this manner, selection could have produced lake whitefish population genotypes optimal to the conditions prevalent in each refugium.

Subsequent to disappearance of the major ice sheets, lake whitefish stocks in the two refugia were provided with ample opportunity, through late-glacial and postglacial watercourses and lakes, to distribute themselves over most of northern North America. This process has been fairly rapid compared to the period of isolation, and in a small way probably continues to present times. Most of central Canada has been crisscrossed with interconnecting watercourses, apparently allowing thorough intermixing of the two presumed stocks of lake whitefish. The rather uniform distribution of the biochemical genetic characters used in this study, together with data on distribution of population modes of gillraker number (Lindsey et al. 1970), in central Canadian lake whitefish supports this contention. For most central Canadian lake whitefish populations, postglacial selection seems to have been unimportant. This does not mean that in terms of the characters studied no selection has been or is acting on central Canadian lake whitefish, but rather that the intermixing of populations over wide areas of central Canada probably has been sufficient to wipe out the effects of selection in most locations. Probably in only a few long and well-isolated headwaters

has there been sufficient opportunity for selection to become important. Thus, lake whitefish populations from peripheral drainages, such as the headwaters of the Saskatchewan, Peace, and Athabasca rivers, small isolated headwater lakes (e.g. Clear and Steeprock lakes in Manitoba and Cache Lake in Northwest Territories) and the early isolated Fraser River drainage show gene frequencies different from those of the main waterways of central Canada. Just such populations also are subject to change, in terms of colonization, genetic drift in small populations, and selection (e.g. in periods of climatic extreme). In some of these lakes (e.g. Clear and Steeprock lakes), large deviations in gene frequencies from populations in lakes in the surrounding area are seen.

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Appendix 1

Gene frequencies (G-3-PDH a^1, b^1, b^2, and b^3 alleles and *ldhH*α^4 allele), sample size, and geographic and climatic data for 38 collections of lake whitefish. N_1 = N for G-3-PDH analysis; N_2 = N for LDH analysis. MJT = mean July air temperature (degrees Celsius); F( ) = frequency of allele in parentheses.

Lake	Lat. (°)	Long. (°)	Elev. (*m*)	MJT	F(a^1)	F(b^1)	F(b^2)	F(b^3)	F(α^4)	N_1	N_2
				Yukon							
Alsek system											
1. Aishihik L.	61.50	137.25	914.7	12.8	0.000	0.000	0.815	0.185	0.000	62	—[a]
2. Dezadeash L.	60.50	137.00	702.6	12.8	0.040	0.000	0.444	0.556	0.000	63	63
Yukon system											
3. Fox L.	61.25	135.50	786.4	13.9	0.000	0.000	0.648	0.360	0.000	43	—
4. Kluane L.	61.17	138.50	781.2	12.8	0.000	0.000	0.357	0.643	0.000	14	—
5. Laberge L.	61.00	135.08	627.9	13.9	0.148	0.000	0.704	0.296	0.000	27	30
6. Mayo L.	63.78	135.33	670.6	15.0	0.166	0.000	0.722	0.278	0.000	18	—
7. McLintock L.	60.58	133.92	762.0	13.9	0.050	0.000	0.733	0.267	0.000	30	—
9. Snafu L.	60.18	133.43	762.0	13.9	0.000	0.000	0.795	0.205	0.000	22	—
9. Squanga L.	60.47	133.63	789.4	13.9	0.223	0.000	0.382	0.618	0.000	251	58
10. Tatchun L.	62.28	136.12	457.2	13.9	0.000	0.000	0.481	0.519	0.000	26	—
11. Teenah L.	60.30	133.42	762.0	13.9	0.232	0.000	0.432	0.568	0.000	158	—
12. Little Teslin L.	60.48	133.48	762.0	13.9	0.139	0.000	0.278	0.722	0.000	18	—
Liard system											
13. Cache L.	61.95	128.23	1295.4	13.9	0.000	0.000	1.000	0.000	0.814	21	24
14. Frances L.	61.25	129.30	774.2	15.0	0.011	0.000	0.831	0.169	0.000	59	29
15. Watson L.	60.10	128.80	679.7	15.0	0.000	0.000	0.978	0.022	0.000	23	27
				Non-Yukon							
Fraser system											
16. Lac la Hache	51.82	121.50	807.7	16.1	0.000	0.000	0.300	0.700	0.674	80	83
17. Williams L.	52.12	122.08	566.6	16.1	0.000	0.000	0.591	0.409	0.136	22	22
Peace system											
18. Moberly L.	53.82	121.75	691.9	16.1	0.007	0.000	0.535	0.465	0.908	71	71
19. Summit L.	54.28	122.67	705.6	15.0	0.000	0.000	0.792	0.208	0.990	48	48
Athabasca system											
20. Talbot L.	53.08	118.00	1001.9	13.9	0.000	0.000	0.740	0.260	0.886	48	48
21. Athabasca L.	59.42	110.00	213.1	18.3	0.024	0.397	0.183	0.420	0.825	63	63
Mackenzie system											
22. Alexie L.	62.68	114.07	221.0	15.0	0.011	0.444	0.211	0.345	0.986	45	37
23. Baptiste L.	62.72	114.23	209.1	15.0	0.000	0.180	0.330	0.490	0.972	50	53
24. Chitty L.	62.72	114.12	222.2	15.0	0.012	0.329	0.354	0.317	0.987	41	38
25. Drygeese L.	62.75	114.17	217.0	15.0	0.019	0.154	0.019	0.827	0.875	107	104
26. Great Slave L.	62.00	114.00	157.9	15.0	0.000	0.364	0.272	0.364	0.896	22	24
South Saskatchewan R.											
27. Waterton L.	49.05	113.90	1278.9	13.9	0.000	0.625	0.025	0.350	0.800	20	20
North Saskatchewan R.											
28. Big Athapap.	54.57	101.42	291.4	16.1	0.006	0.281	0.303	0.416	0.950	160	50
29. Little Athapap.	54.62	101.67	291.4	16.1	0.013	0.397	0.359	0.244	0.977	39	43
30. Clearwater L.	54.00	101.55	260.6	18.3	0.000	0.411	0.191	0.398	0.950	143	30
31. Narrows (L. Athapapuskow)	54.60	101.45	291.4	16.1	0.023	0.386	0.136	0.478	0.983	22	29
32. Wabamun L.	53.53	114.58	725.4	17.2	0.037	0.046	0.630	0.324	0.882	54	55
33. North L. Winnipeg	53.00	98.00	217.3	18.3	0.018	0.225	0.311	0.464	0.949	111	108
34. Clear L.	50.67	100.00	614.8	18.3	0.000	0.381	0.131	0.488	0.714	80	77
35. Steeprock L.	52.58	101.42	701.0	18.3	0.019	0.250	0.317	0.433	0.135	52	52
Churchill system											
36. Crean L.	54.08	106.17	524.3	16.1	0.000	0.091	0.114	0.795	0.955	22	22
37. Churchill R. mouth	58.83	94.17	0	12.2	0.014	0.429	0.243	0.328	0.957	35	46
38. Primrose L.	54.92	109.75	598.6	16.1	0.000	0.133	0.400	0.467	0.933	30	30
Totals										2200	1384

[a]Yukon populations were considered homozygous for *ldhH*α^B allele after an unbroken run of 151 fish from three areas because probability of encountering the other allele by further sampling had dropped to < 5%.

Methoxychlor Residue Studies in Caged and Wild Fish from the Athabasca River, Alberta, Following a Single Application of Blackfly Larvicide

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On June 4, 1974, the Athabasca River was treated with methoxychlor for control of blackfly larvae. Pesticide was introduced into the water in a manner calculated to generate a 15-min pulse of treated water containing 300 μg methoxychlor/ℓ. A preliminary experiment with rainbow trout (*Salmo gairdneri*) indicated that liver or kidney residues of methoxychlor would be suitable for monitoring likely times and places to expect toxic effects on fish in the river. Fish (*Salmo gairdneri*, *Catostomus commersoni*, *C. catostomus*, and *Hybopsis gracilis*) were caged in order to observe residues and mortality. Caged fish were not killed by methoxychlor but they contained lower liver residues than wild fish of the same species captured near cages. Contaminated wild fish were found upstream of the treatment site after treatment. Residues were below detection limits by June 16. Residue measurements suggested that the most likely times to expect fish poisoning were the 2 wk following treatment and that the most likely places were those river areas extending a few kilometres in both directions from the point of application. Evidence of fish movements and low pesticide residues in caged individuals reduced confidence in residue methods as monitoring tools.

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Le 4 juin 1974, la rivière Athabasca fut traitée au méthoxychlore dans le but de contrôler les larves de simulies. On introduisit le pesticide dans l'eau de manière à engendrer une pulsation de 15 min d'eau traitée contenant 300 μg de méthoxychlore/ℓ. Une expérience préliminaire effectuée sur la truite arc-en-ciel (*Salmo gairdneri*) indique que les résidus de méthoxychlore du foie ou du rein conviendraient à la surveillance des temps et des endroits probables où trouver des effets toxiques sur les poissons dans la rivière. On a placé des poissons (*Salmo gairdneri*, *Catostomus commersoni*, *C. catostomus* et *Hybopsis gracilis*) dans des cages afin d'observer les résidus et la mortalité. Les poissons en cage ne sont pas tués par le méthoxychlore mais contiennent de plus bas niveaux de résidus hépatiques que les poissons sauvages de la même espèce capturés près des cages. On a trouvé des poissons sauvages contaminés en amont du site de traitement après le traitement. Au 16 juin, les résidus sont inférieurs aux limites de détection. Les mesures de résidus suggèrent que le temps où l'on peut s'attendre le plus à un empoisonnement chez les poissons est les 2 semaines qui suivent le traitement, et que les endroits les plus probables sont ces secteurs de la rivière compris dans les quelques kilomètres en aval comme en amont du point d'application. Le déplacement des poissons et les faibles résidus de pesticide chez les individus en cage font qu'on accorde moins de confiance aux méthodes des résidus comme outils de surveillance.

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BLACKFLY control programs have been carried out in western Canada for many years by injecting insecticides into flowing waters where larvae develop (Fredeen et al. 1971). In the late 1960s methoxychlor replaced DDT as the insecticide of choice (Fredeen 1974; Fredeen et al. 1975) and efforts are still underway to understand the nature and severity of its effects upon nontarget stream fauna (Fredeen 1975; Wallace and Hynes 1975; Eisele and Hartung 1976). A review of effects of methoxychlor upon environmental quality has been published by the National Research Council (Gardner and Bailey 1975).

The Athabasca River had not been treated with insecticide prior to an experimental spring treat-

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ment in 1974. Methoxychlor was introduced into the river from a bridge at Athabasca, Alberta, so that a 15-min pulse of treated water theoretically containing 300 μg methoxychlor/ℓ was generated. Treatment took place at dawn on June 4, 1974 under the supervision of Dr K. R. Depner, Agriculture Canada, Lethbridge, Alberta.

We were unable to predict whether fish would be poisoned following treatment, and the Athabasca river, with no history of previous larviciding, offered opportunity to observe effects of a single treatment. Among our measurements were concentrations of methoxychlor in fish tissues and this report presents those concentrations. We were not seeking to document a public health hazard caused by accumulation of methoxychlor in edible portions of fish. Rather, we were interested in residue concentrations as indicators of risk to the fish containing them. For this purpose nervous tissue might be the best tissue to analyze, but it was not available in quantity, and we report an experiment leading to our selection of liver.

Methods to monitor effects of pesticides in large rivers are not well developed. (On May 29, 1974 the Athabasca River at Athabasca was about 200 m wide with a volume discharge rate of 1008 m^3/s, Beltaos and Gerard 1975.) Useful techniques such as blocking seines are successful in small streams but simply cannot be applied to large turbulent rivers. We chose to measure residues in fish captured wild in the river, in fish placed in cages in the river, and in fish treated experimentally in pools in Winnipeg. Each of these measurements has inherent limitations but we hoped that each method would serve to check another method and that together they would allow a reasoned judgement of the risk of poisoning fish during blackfly control.

Materials and Methods

Fish obtained from four sources were analyzed for methoxychlor in liver and sometimes in other tissues. The sources were: rainbow trout (*Salmo gairdneri*) from the Freshwater Institute's Rockwood (Manitoba) hatchery, rainbow trout provided by the Alberta Department of Lands and Forests, white and longnose suckers (*Catostomus commersoni* and *C. catostomus*), captured with dipnets in Flat Creek, Alberta, and native Athabasca River fish captured with hoopnets at points in that river.

A group of yearling rainbow trout from the Rockwood hatchery was exposed to methoxychlor in an outdoor pool under conditions described earlier (Lockhart et al. 1973). A single injection of 1.34 g methoxychlor (DuPont of Canada) in 120 ml ethanol was mixed into a polyethylene-lined pool containing a constant volume of 4450 ℓ of water flowing at 15 ℓ/min. Under these conditions, the nominal concentration, assuming complete mixing, at any time can be calculated using the expression

$$C_t = C_0 \exp\left(-\frac{\text{(flow rate)}}{\text{(pool volume)}}\, t\right)$$

where C_t = concentration at any time t (minutes); C_0 = initial concentration upon mixing at $t = 0$; t = time since mixing (minutes).

Comparing calculated and measured concentrations of methoxychlor in water during experimental poisoning of rainbow trout, it seems that the fish probably experienced only about one quarter of the anticipated dose, as shown below.

Time after mixing (*min*)	Calculated conc = ($\mu g/\ell$)	Measured conc = ($\mu g/\ell$)
0	300	93.0
15	285	75.0
33	268	116.0
60	245	51.0
120	200	55.0
403	77	7.3
1458	2	0.5

Water temperature was 19–20°C and fish were not fed during the exposure. Groups of fish were withdrawn at intervals from 15 min to 24 h after adding methoxychlor, and residue concentrations were determined in liver, kidney, muscle, and gut with associated visceral fat.

A second group of yearling rainbow trout was provided by the Alberta Department of Lands and Forests, and eight fish were placed in each of 12 1.27-cm wire mesh cages measuring 10 × 20 × 20 cm, which were placed in the Athabasca River a few minutes prior to methoxychlor treatment. Three cages were anchored near shore, approximately 200 m upstream from the treatment point, and the remaining nine were suspended from a boat and allowed to drift approximately 3 min behind the treatment front as marked by several hundred oranges. We hoped that fish drifting with the pulse of treated water would experience the maximum exposure to be experienced by a wild fish. Cages were removed at various times from 15 min to 101 h after treatment and of the eight fish in each, four were dissected to yield pooled samples of liver, kidney, muscle, and digestive tract with visceral fat and the remaining four were wrapped individually in foil for whole-body analyses. The first three treated cages (15, 30, 60 min) were removed from the water while drifting and the remaining six were anchored nearshore at a point some 5 km downstream from the treatment site. One cage was removed at each time 3, 6, 12, 28, 59, and 101 h after treatment. All trout cages, upstream and downstream, were examined daily in a check for mortality.

Native species of fish (white suckers, longnose suckers, and flathead chub, *Hybopsis gracilis*) were captured in the Athabasca River and in Flat Creek (Athabasca drainage) and these were placed in wire cages. Cages of 2.54-cm galvanized wire mesh were made by rolling 123-cm wide mesh into cylinders of 76 cm diameter and by enclosing each end with a

TABLE 1. Methoxychlor concentrations in rainbow trout, *Salmo gairdneri*, (in micrograms per gram wet tissue) at intervals after exposures of up to a theoretical 300 $\mu g/\ell$. Figures are means of values from four fish with standard deviations.

Time after Treatment (*h*)	Liver $\bar{x} \pm$ SD	Digestive tract + fat $\bar{x} \pm$ SD	Muscle $\bar{x} \pm$ SD	Kidney $\bar{x} \pm$ SD
Pretreatment	<0.02	<0.02	<0.02	<0.02
0.25	9.10±3.84	2.62±1.33	1.00±0.17	4.60±1.43
0.50	16.9±5.99	5.12±3.45	1.29±0.42	10.4±5.24
1.0	15.7±3.86	9.35±0.78	3.42±0.41	9.83±2.07
2.0	24.9±9.21	9.34±3.04	4.30±0.51	19.3±7.38
2.17 (moribund)	29.6±8.85	17.3±3.46	7.64±2.22	20.3±5.86
6.0	13.7±5.74	34.5±10.75	9.81±2.01	14.9±2.16
24.0	2.61±0.65	55.1±7.58	4.67±3.40	5.89±2.16

circular section of mesh. Four cages were situated at each of four sites (2.5 km upstream, and 4.5, 33.5, and 74.5 km downstream) and 6–11 fish (depending upon availability) were placed in each cage. At one site (4.5 km downstream) white suckers, longnose suckers, and flathead chub were caged together since we hoped to determine which would best withstand caging; at other sites white suckers only were used. Cages were all stocked by June 1, 1974 and they were sampled by removing one cage from each site on June 5, 11–12, 18–19, and July 9–10. Fish were not fed artificially during the experiment. Upon removal from the water, fish in each cage were bled, weighed, measured, and dissected; tissues were wrapped in foil and frozen for analyses.

Wild fish were captured with hoopnets set near cages at three sites (2.5–4.5 km upstream, 4.5 and 74.5 km downstream) and they were treated in the same manner as caged fish.

Methoxychlor in fish tissues was measured by gas–liquid chromatography using a Tracor MT-220 gas chromatograph equipped with an electron-capture detector (Solomon and Lockhart 1977). Samples were ball-mill extracted (Grussendorf et al. 1970) and cleaned up either on Florisil® alone or by lipid freeze-out followed by Florisil®. Cleaned up extracts in *n*-hexane were analyzed on a short gas–liquid chromatography column with a retention time of about 2.2 min.

Results and Discussion

Uptake of methoxychlor by rainbow trout exposed in Winnipeg was remarkably rapid, especially into liver (Table 1). Such rapid uptake into deep tissues like liver and kidney by non-feeding fish implies distribution by the vascular system after uptake at some site, presumably gills (Fromm and Hunter 1969). Of the four tissue residues examined, concentrations in livers of the moribund group removed from the water after 2.17 h of exposure were highest, and because of that, we chose to monitor residues in liver tissue of Athabasca River fish. Kidney concentrations of methoxychlor were also high in the moribund group, and concentrations in liver and kidney related better to symptoms of gross morbidity (rapid movements, taking air at surface, then loss of equilibrium) than did those in muscle or digestive tract with fat deposits. By 6 h after starting exposure, liver and kidney residues were declining; however, muscle was at its peak concentration, and digestive tract was still increasing. Fish displayed no sign of gross morbidity at this and the final sample collection at 24 h, and recovery was indicated by several weeks' posttreatment observation.

This experiment shows that muscle residues were apparently not a suitable indicator of toxicity to rainbow trout, and their value in monitoring may well be restricted to judging the quality of fish as human food as suggested by Holden (1973). Furthermore, the tissue distribution was highly sensitive to the time elapsed since starting treatment; considering relative tissue weights the group at 6 h may well have had the highest body burden of methoxychlor, although it was most obviously toxic at 2.17 h. Our choice of liver tissue seems defensible considering the use we had in mind for the data – as a tracer for determining when and where in river areas to expect toxicity. Clearly, if the question being asked concerned terminal tissue residues, then liver tissue would not be suitable.

Residues in yearling rainbow trout caged in the Athabasca River are all strikingly low as compared with the Winnipeg experimental exposure (Table 2). These trout accumulated virtually no methoxychlor during the first 3 h – time partially spent drifting behind the front of the treatment. During the period between 3 and 6 h, however, when cages were anchored on the bottom below a riffle near the west bank, the greatest rate of

TABLE 2. Methoxychlor concentrations in yearling rainbow trout (in micrograms per gram wet tissue) caged in the Athabasca River, Alberta, at intervals after treating the river with methoxychlor at 300 $\mu g/\ell$. Tissues dissected from four fish were pooled to yield composite samples for tissue analyses. Four whole fish were also analyzed from each cage and the whole-fish column gives the range of concentrations found.

Time after treatment (*h*)	Liver	Gut with fat	Muscle	Kidney	Whole fish
0.25[a]	0.09	<0.02	0.06	0.04	<0.02–0.04
0.50[a]	0.19	0.04	0.05	0.15	0.04–0.10
1.0[a]	0.08	0.05	0.04	0.07	0.04–0.06
3.0	0.16	0.06	0.04	0.18	0.03–0.06
6.0	0.96	0.39	0.30	0.86	0.60–1.56
12.0	1.34	0.58	0.07	0.64	0.69–1.08
28.0	0.34	0.20	0.29	0.28	0.23–1.75
59.0	0.08	0.72	0.08	0.08	<0.02–0.75[b]
101.	0.03	<0.02	<0.02	<0.02	<0.02–0.71[b]

[a]Time spent drifting with the pulse of treated water.
[b]Three fish represented (one lost, one dead).

accumulation took place. Evidently, a source of methoxychlor existed some 3–6 h after treatment, only 5 km from the treatment point, indicating a slow-moving source, presumably due to low water velocity near the edge of the river (Beltaos and Gerard 1975). (Pesticide concentrations in water and sediment have been measured by Dr W. A. Charnetski, Agriculture Canada, Lethbridge, Alberta). The data for pooled individual tissues from four fish and those for whole-body concentrations for the other four fish in each cage agree well up to about 12 h; after that, particularly at 101 h the whole-body residues were sometimes much higher than expected from the tissue residues, raising the possibility of an important tissue reservoir in addition to those analyzed.

Caged trout provided an on-site "bioassay" of a sort, and their survival confirmed the Saskatchewan River cage study reported by Fredeen (1974). Only four trout died over the 4-day period, three in upstream cages and one downstream. If this "bioassay" had relevance for the native river fish fauna, then treatment was not toxic to fish over at least the first 5 km. Some difficult questions do present themselves in the data of Table 2. Why, for example, did the fish take up so little methoxychlor during the first 3 h? During dissection we noted that these yearling trout had virtually no fat among pyloric ceca, and further, that fish had not fed for some time as indicated by presence of only occasional small stones in digestive tracts. Fish with little fat may accumulate methoxychlor less efficiently than those with large amounts of fat; Fredeen et al. (1975) have used this reasoning to explain the uptake of methoxychlor by goldeye in the Saskatchewan experiments. Water temperature differences may partially explain the lower net accumulation in the field since river temperature was only 13–14°C, as compared with 19–20°C in the Winnipeg experiment. Macek et al. (1969) reported methoxychlor to be more toxic to fish in cool water (12.7°C) than in warm water (18.3 and 23.8°C). We carried out a cold-water exposure (2.3–6.7°C) of two rainbow trout (survivors from a previous DDE exposure) during March 1974, in the same manner as described in Methods. Both trout died sometime between observations at 5 and 23 h; liver residues were 13.8 and 21.2 $\mu g/g$, values well above those recorded in the field, and apparently temperature cannot be the sole explanation for low residues in the river exposure.

Fredeen et al. (1975) reported methoxychlor binding to silt particles in the Saskatchewan River. Uptake from silt particles, although possible (Zitko 1974), may be less efficient than uptake of dissolved material (solubility 0.1 mg/ℓ at 25°C). Variation in whole-fish data of Table 2 became noticeable at 28 h and very large at 59 and 101 h, giving rise to speculation that different individuals may metabolize or excrete methoxychlor at very different rates; if differences were confined to the uptake rates, then we might have expected large discrepancies at the end of the 6- and 12-h samples as well.

Information from cages of native Athabasca River species tended to confirm the experience with caged trout. At no time were residues detected in fish caged upstream from the treatment point, indicating satisfactory sample processing, storage, and analyses without contamination. Methoxychlor had reached fish at all three down-

stream cage sites by June 5, the day after treatment, but liver residues were very low in suckers. A single flathead chub in a cage 4.5 km downstream had the highest liver residue at 0.47 μg/g, close to the 28-h value of 0.34 μg/g found in trout caged nearby (Table 2). On June 11–12, when caged fish were sampled a second time, all were still alive, and residues were found only in two flathead chub at the same 4.5-km site. On the third sampling, June 18–19, a chub at that site and a white sucker at 35.5 km downstream contained liver residues at 0.03 and 0.04 μg/g, respectively, and by the final cage sample, July 9–10, no liver residues were found.

In general, the cages of native species again served as bioassays, and survival argues for safety of the larviciding operation, at least in terms of acute fish mortality. Methoxychlor was not found in white suckers caged 4.5 km downstream, even though it must have been available to them, since it was found in longnose suckers and chub occupying the same cages. Of the three native species caged, none was completely suitable in these cages; both species of suckers tended to injure themselves in the head region, apparently by striking the wire mesh, and some chub were found caught in the mesh squares, as in a gillnet. Smaller mesh or a cage lining of flexible mesh seems likely to help.

The caged fish were compared with wild fish captured by setting hoopnets near cages (Table 3) and results were strikingly different. Residue levels in free fish were generally higher than those in the same species caged, although sampling times were somewhat different. The highest liver residue found in a free fish was 9.64 μg/g in a chub captured 4.5 km downstream on June 8, as compared with a maximum of 0.79 μg/g for that species caged. Similarly, the highest value for white sucker liver was 0.75 μg/g in a wild fish upstream on June 8; that for a caged white sucker was 0.11 at the site 35.5 km downstream on the day after treatment. Longnose suckers contained slightly more methoxychlor in liver than did white suckers, peak values for free and caged individuals being 0.94 and 0.07 μg/g, respectively. Very few other species of fish were caught after treatment. A few northern pike (*Esox lucius*), walleye (*Stizostedion vitreum vitreum*) and goldeye (*Hiodon alosoides*) were caught, with traces of methoxychlor found only in a single pike. In the Saskatchewan River study (Fredeen et al. 1975), differences among fish species were noted with goldeye accumulating most methoxychlor; here species differences are also evident, but flathead chub were the most highly contaminated of those sampled. Greater concentrations were found in caged chub than in caged white or longnose suckers in the same cages; this greater accumulation in chub livers was also found in wild fish, and the cage data appear valid for comparisons among these species. Unfortunately, caged fish did not contain liver residues as great as those of free wild fish of the same species; hence, caged individuals were not comparable with wild ones of the same species. Therefore, survival of caged fish must have provided an underestimate of risk to wild ones.

Several hypotheses may be advanced to explain reduced accumulations by caged fish: perhaps caged fish were in areas of incomplete mixing and never experienced the anticipated concentration of methoxychlor; perhaps caged fish were starving and did not have access to a food component of methoxychlor uptake; perhaps caged fish were lean and did not accumulate methoxychlor readily; perhaps caged fish were protected from currents by the cages, and consequently their work load was lower, and they forced less water across gills for respiration. Whatever the reason for differences among caged and free fish of the same species, reliance upon cage bioassays in Athabasca studies seems unacceptable.

Perhaps the most noteworthy observation was the appearance of residues in suckers and chub captured at the upstream site, some 4.5 km upstream from treatment. This presumably indicates an upstream movement by these species, and we may wonder whether this movement represents normal migratory behavior or some response to treatment. An aspect of the residue data which seems readily amenable to experimental analyses, but which has not been reported, is the apparently low residue accumulation by fish 74.5 km downstream. We did not find methoxychlor in any of the wild fish captured there and exposure for longer times to anticipated lower downriver concentrations apparently resulted in less liver contamination. This argument has some support from laboratory toxicology. For example, Katz (1961) found the median tolerance limit for rainbow trout to be 62.6 μg/ℓ in tests running 24, 48, 72, and 96 h. The longer exposure time at 96 h was not associated with a compensating reduction in methoxychlor required. Similarly, Merna et al. (1972) presented a curve of toxicity against exposure time and toxicity was virtually insensitive to exposure after about 6 days.

Since the last measurable liver residues were found on June 16, a period of about 2 wk after treatment are apparently required to monitor fish for toxic effects. Furthermore, the stretches of

TABLE 3. Methoxychlor residues in native fish from the Athabasca River at intervals after water treatment on June 4, 1974 for blackfly control. WS = white sucker; LS, longnose sucker; C, flathead chub; P, northern pike; W, walleye.

Sample date	Upstream		Downstream 4.5 km		Downstream 74.5 km	
	Species	Methoxychlor	Species	Methoxychlor	Species	Methoxychlor
June 5			WS	<0.02	WS	<0.02
			WS	0.08	P	<0.02
			C	0.15	P	<0.02
			C	<0.02		
			C	0.33		
			P	0.05		
June 6	WS	<0.02	WS	0.10		
	WS	0.10				
June 7	WS	0.66				
	WS	<0.02				
	WS	0.03				
	WS	0.03				
	LS	0.69				
June 8	WS	0.75	C	9.64		
	WS	<0.02				
	LS	0.72				
	LS	0.22				
	LS	0.12				
June 9	WS	<0.02	WS	0.30		
	LS	0.68	WS	0.43		
	LS	0.37	LS	0.43		
	P	<0.02	LS	0.04		
			LS	0.13		
			LS	0.94		
			C	2.62		
			C	2.59		
			C	3.01		
June 10	LS	<0.02	LS	0.15		
	LS	0.23	LS	0.70		
	W	<0.02				
June 11					7 WS	
					2 P	all <0.02
June 12	WS	<0.02	WS	<0.02		
	C	3.14				
June 16	3 WS		C	<0.02		
	2 LS		C	0.04		
	1 W,	all <0.02	C	1.18		
June 19–Aug. 12	26 samples analyzed, various sites and species, all <0.02.					

river for the first few kilometres in both directions from the treatment point seem to be areas of greatest interest.

Hazards of organochlorine compounds to vertebrates often involve aspects of reproduction, and we have no measure of reproduction in Athabasca River fish populations. Flathead chub had not begun spawning at the time of treatment and ripening ovaries were analyzed from 10 females collected between June 5 and June 16, all with measurable liver residues. Methoxychlor in ovaries varied from 0.03 to 9.33 μg/g (liver from 0.11 to 9.64 μg/g) and the correlation coefficient between liver and ovarian residues was +0.90 ($P < 0.01$). Values around 5 μg/g total DDT in eggs have been associated with the onset of

reproductive failure in lake trout (Burdick et al. 1964). Some of the ovarian methoxychlor concentrations in flathead chub exceeded 5 μg/g; however, it is not known whether they are objectionable either at the time they are present or for subsequent events in larval development.

The major value in the data presented here may lie in the inconsistencies revealed by them. The Winnipeg experimental rainbow trout contained much more methoxychlor than the caged trout; the caged trout did not take up methoxychlor when they were expected to; the caged suckers and chub contained much less liver methoxychlor than wild suckers and chub; at least some wild fish had moved unknown distances upstream between times of treatment and capture. Cage bioassays seem to have systematically underestimated risk of fish poisoning; capture of wild fish selects survivors (unless driftnets are used) and their unknown history makes interpretation of residues difficult. Overall, methods of cage bioassays and residue chemistry, as practised by ourselves and others, seem to have as great a capacity to mislead as to inform.

Biological and biochemical aspects of the fish used here for residue analyses will be presented separately (Lockhart and Metner unpublished data).

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Effect of Temperature and Feeding Rate on the Liver–Somatic Index of the Largemouth Bass, *Micropterus salmoides*[1]

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At a controlled food intake level after 14 days at a given feeding regime, the liver–somatic index (liver weight as a percentage of body weight) of largemouth bass, *Micropterus salmoides*, was positively correlated with daily food intake. At temperatures of 20.0 and 28.0°C there was no overlap in the ranges of the liver–somatic index of bass fed 0, 2, and 4% of their body weight in live green sunfish (*Lepomis cyanellus*) per day. Within a given feeding regime, increased temperature lowered the liver–somatic index.

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A un niveau contrôlé de quantité de nourriture absorbée après 14 jours d'un régime alimentaire donné, l'indice hépato–somatique (poids du foie en pourcentage du poids corporel) de l'achigan à grande bouche, *Micropterus salmoides*, a une corrélation positive avec la quantité de nourriture absorbée quotidiennement. Aux températures de 20.0 et 28.0°C, il n'y a pas chevauchement des gammes d'indices hépato–somatiques d'achigans nourris de 0, 2 et 4% de leur poids corporel de crapets verts (*Lepomis cyanellus*) vivants par jour. Avec un régime alimentaire donné, une élévation de température résulte en un abaissement de l'indice hépato–somatique.

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KNOWLEDGE of the adequacy of forage supply is basic to the successful management of largemouth bass, *Micropterus salmoides*, populations. Several methods, including inventory of the forage population, age and growth studies, examination of stomach contents, and coefficient of condition are currently used to obtain this information. All of these methods require massive data and each has certain other specific weaknesses: inventory of the forage requires supportive information on the density of the bass population and the availability of the forage as affected by size and susceptibility to capture; age and growth studies are useful in determining prior history of food intake, but not the short-term current intake, which usually is the information most useful in management; the value of analysis of stomach content is limited by a lack of a complete understanding of the feeding response of the largemouth bass; and coefficient of condition, which measures the plumpness of a fish, is a convenient but not highly sensitive method and its value for management is questionable.

The present aquaria study investigated the potential of the liver–somatic index (wet weight of the liver divided by the weight of the fish) as a measure of current food intake. Many fish are known to store significant amounts of fats and glycogen in the liver (Hoar and Randall 1971). Changes in the quantity of fat, glycogen, and water stored in the liver have been shown to cause a significant change in liver weight in at least some species. Phillips et al. (1960) demonstrated such a relationship in the brook trout (*Salvelinus fontinalis*), and Kott (1971) in the sea lamprey (*Petromyzon marinus*). Judd and Cross (1966) indicated a relationship between season and liver weight in fed channel catfish (*Ictalurus punctatus*). Other investigators have found that temperature influences liver weight. Thus, Dean and Goodnight (1964) found that liver glycogen in largemouth bass increased at

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low temperatures. Since these studies have shown liver weight to be a function of temperature, in order to investigate the hypothesis that the liver–somatic index can be used as a measure of current food intake, this study compared liver–somatic indexes at various levels of food intake at different temperatures.

Materials and Methods

Fourteen-day feeding trials were conducted with largemouth bass under each of three temperature conditions, at three feeding rates. At the conclusion of the 14-day trials, the bass livers were analyzed for moisture content, crude fat, crude protein, and glycogen.

The feeding trials were conducted at water temperatures of 14.0, 20.0, and 28.0°C. The 14.0°C trials were conducted in 132-ℓ, covered, concrete aquaria. One bass was placed in each aquarium. For the trials at 20.0 and 28.0°C, 200-ℓ, covered, glass aquaria were used. Two bass were placed in each aquarium and separated by a divider made of stainless steel screen. All aquaria in the study received 450 ± 25 mℓ/min of charcoal filtered city water. Thus, the water exchange rate was 3.2 times/24-h period for the glass aquaria and 4.9 times/24-h period for the concrete aquaria.

Experimental procedures were similar for all three temperature trials. At the beginning of each trial, 20 bass that ranged in total length from 24 to 31 cm (approximately 225 g) were obtained from Lake Chautauqua near Carbondale, Illinois, by electrofishing in September, October, and November 1974. At the time of collection, surface temperatures in Lake Chautauqua were within 5 deg C of proposed experimental temperatures. Within 3 h after capture, five bass were killed and their standard length (±1 mm), wet weight (±0.1 g) and wet liver weight (±0.001 g) determined. The liver–somatic index was calculated by the following formula:

$$\frac{\text{wet wt liver(g)(}-\text{gall bladder)} \times 100\%}{\text{wet wt fish(g)(including liver)}-\text{wet wt gonads(g)}}$$

A closely related term, liver condition, is in the literature; however, since the liver-condition formula as used by Graham (1923) and later by Jangaard et al. (1967) does not substract the weight of the gonads from body weight, the term liver condition is not appropriate in this study. Thus, liver–somatic index is used.

Before the trials the bass were held in acquaria at the experimental temperature for 7 days. During this conditioning period the bass were offered green sunfish (*Lepomis cyanellus*) at 1% of their body weight per day. Subsequently, the 15 bass of each temperature trial were randomly divided into three groups and each group was fed for 14 days at a specific rate. For the 14.0°C trial, five bass were not fed, five bass were fed 0.5% of their body weight per day, and five bass were fed 1% of their body weight per day, the maximum they would consume at this low temperature. For the 20.0 and 28.0°C trials, five bass were not fed, five bass were fed 2% of their body weight per day, and five bass were fed 4% of their body weight per day.

At the end of the 14-day feeding period, all 15 bass were killed 24 h after their last feeding. The fish were anesthetized with quinaldine and weighed to the nearest 0.1 g. With a pair of scissors, the body cavity was opened from the anus to the branchiostegal rays. The liver and gonads were removed with forceps, blotted dry, and weighed immediately. The wet liver weight and the gonad weight were obtained to the nearest 0.001 g, and sex was determined.

The livers were analyzed for moisture content, crude fat, crude protein, and glycogen. Immediately after weighing, one half of the wet liver was placed in a capped glass vial and quick frozen in acetone on dry ice for 2 min. The liver samples were then placed in a freezer at −30°C until they were analyzed for glycogen. The remaining liver halves were individually dried and moisture content was determined for each.

However, because of their small size, the five livers from each feeding regime were combined and analyzed for crude fat, crude protein, and glycogen. Duplicate chemical analyses were made for each pooled feeding rate–temperature group. Moisture, nitrogen, and crude fat (ether extract) content were determined according to the procedure of A.O.A.C. (1970). Glycogen was determined according to Seifter et al. (1950).

To provide information on the nutritional value of the food, a sample of green sunfish that served as forage in each feeding trial was analyzed for moisture content, crude fat, crude protein, and caloric content. Caloric content was determined with a Parr adiabatic bomb calorimeter.

Results

After the 14-day feeding trial, the liver-somatic index of the bass was positively correlated with daily food intake. Since there was no significant difference (0.05 probability level) between the average weight of fish within each feeding rate, this correlation was not due to allometric growth of the liver. Within the 20.0 or 28.0°C trials there was no overlap in the range of liver–somatic indexes at the three feeding rates (Table 1); within the 14.0°C trial there was overlap in the range of liver-somatic indexes at the different feeding rates. Paired comparisons between feeding rates in the 14.0°C trial, using Tukey post hoc analysis ($P < 0.05$), showed no significant difference in the mean liver–somatic index between 0 and 0.5%, and 0.5 and 1% feeding rates. There was a significant difference between 0 and 1% feeding rates. When the gonad weights were added to the total body weights, the recalculated liver–somatic indexes decreased a maximum of 0.5% of their original values.

TABLE 1. Liver–somatic index and coefficient of condition (K) of largemouth bass, *Micropterus salmoides*, at selected temperatures and feeding rates at the end of each 14-day experimental trial.

Mean trial temp (°C)	Daily feeding rate (%)	No. fish	Liver–somatic index (%)			K		
			Mean	Range	Variance	Mean	Range	Variance
14.0	0	5	0.985[a]	0.884–1.128	0.011	1.99	1.80–2.22	0.03
	0.5	5	1.120[a]	0.996–1.451	0.035	2.00	1.80–2.22	0.03
	1	5	1.284[a]	1.064–1.435	0.022	2.10	1.92–2.21	0.01
20.0	0	5	0.659	0.565–0.779	0.010	1.90[b]	1.72–2.22	0.04
	2	5	1.326	1.086–1.477	0.028	2.21[b]	2.00–2.30	0.01
	4	5	1.955	1.748–2.161	0.025	2.24[b]	2.17–2.34	0.01
28.0	0	5	0.573	0.521–0.628	0.002	1.92	1.85–1.98	0.01
	2	5	0.971	0.896–1.074	0.006	2.06	1.91–2.36	0.04
	4	5	1.402	1.242–1.531	0.012	2.29	2.22–2.43	0.01

[a]Lines in this column indicate paired means that are not significantly different from one another according to Tukey post hoc analysis ($P < 0.05$).

[b]Lines in this column indicate paired means that are significantly different from one another according to Tukey post hoc analysis ($P < 0.05$).

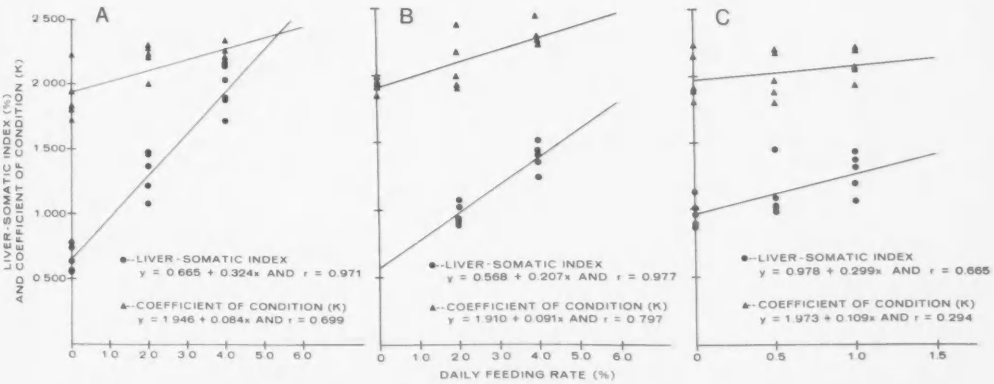

FIG. 1. Linear regression of liver-somatic index and coefficient of condition (K) on daily feeding rate for the mean trial temperature: A, 20.0°C; B, 28.0°C; C, 14.0°C.

As feeding rate increased in each experimental trial the coefficient of condition (K) also increased, but unlike the liver–somatic index, there was considerable overlap in the range of the K values between feeding rates in all three temperature trials (Table 1). According to Tukey post hoc analysis ($P < 0.05$), mean K values were not significantly different at the 2 and 4% feeding rates in the 20.0°C trial and at the 0 and 2% feeding rates in the 28.0°C trial. The mean coefficient of condition (K) was not significantly different between any of the three feeding rates in the 14.0°C trial (ANOVA, $P < 0.05$).

At all three experimental temperatures there was a positive correlation between feeding rate and the final liver–somatic index. The correlation coefficient (r) was 0.971 at 20.0°C (Fig. 1A) and 0.977 at 28.0°C (Fig. 1B), but it was somewhat lower (0.665) for the 14.0°C trial (Fig. 1C). These correlations are somewhat higher than the correlations between feeding rate and final K values (Fig. 1), r values of which were 0.699 at 20.0°C, 0.797 at 28.0°C, and 0.294 at 14.0°C.

At a given feeding rate, as the experimental temperature increased the liver–somatic index decreased (Table 1). When compared at the same feeding rates the liver–somatic index was significantly different between experimental trial temperatures, except between the 20.0 and 28.0°C trials at the 0% feeding rate (Tukey post hoc analysis, $P < 0.05$). In contrast to the liver–somatic index, the final K values were not sig-

TABLE 2. Moisture content, crude fat, crude protein, and caloric content of green sunfish, *Lepomis cyanellus*[a], fed to largemouth bass.

Mean trial temp (°C)	Moisture content (%)	Crude fat (%)		Crude protein (%)		Cal/g fish	
		wet	dry	wet	dry	wet	dry
14.0	77.25	1.22	5.44	14.54	63.83	746	3279
20.0	76.78	1.38	6.02	15.16	65.18	853	3672
28.0	79.16	1.34	6.45	13.46	64.54	792	3802

[a]Weight of green sunfish ranged from 2 to 12 g.

TABLE 3. Chemical analysis of largemouth bass livers at selected temperatures and feeding rates.

Temp (°C)	Daily feeding rate (%)	Moisture content (%)	Crude protein (%)		Crude fat (%)		Glycogen (%)		Estimated[c] (%)	
			wet[a]	dry[b]	wet	dry	wet	dry	wet	dry
14.0	0	74.59	13.59	53.48	0.52	2.05	6.89	27.12	4.41	17.35
	0.5	73.91	11.84	45.37	0.59	2.26	9.18	35.18	4.48	17.19
	1	73.49	11.39	42.97	0.68	2.57	10.79	40.70	3.65	13.76
20.0	0	77.77	15.50	68.07	0.50	2.20	—	—	—	—
	4	73.07	11.93	44.29	0.69	2.56	—	—	—	—
28.0	0	77.45	15.24	67.58	0.67	2.95	2.29	10.15	4.35	19.32
	2	76.21	11.60	48.76	0.77	3.25	6.60	27.75	4.82	20.24
	4	75.64	12.38	50.81	0.83	3.40	7.34	30.13	3.81	15.66

[a]All wet percentage values are based on wet liver weights.
[b]All dry percentage values are based on water free liver weights.
[c]Estimated ash was calculated by adding moisutre content + crude protein + crude fat + glycogen and subtracting this total from 100%.

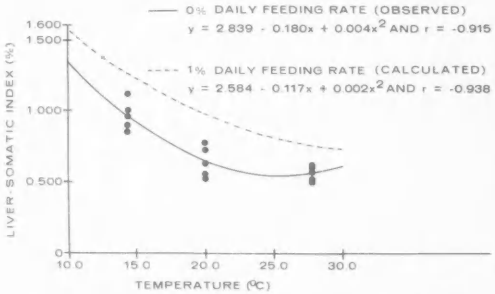

FIG. 2. Curvilinear regression of liver–somatic index on temperature for the 0% and 1% daily feeding rates.

nificantly different between experimental trial temperatures when analyzed at the same feeding rate (ANOVA, $P < 0.05$).

Since the bass would not consume more than 1% of their body weight per day at 14.0°C, it was only possible to obtain a curvilinear regression equation from data for the liver–somatic index and the three experimental temperatures at the 0% feeding rate (Fig. 2). The linear regression equations of liver–somatic index and feeding rate at 20.0 and 28.0°C (Fig. 1A, B) were used to calculate a theoretical liver–somatic index at these temperatures for the 1% feeding rate. These two values, and the experimental value at 14.0°C, were used to determine the curvilinear relationship between liver–somatic index and temperature at the 1% feeding rate. At 0 and 1% feeding rates a negative curvilinear relationship was found between liver–somatic index and temperature (Fig. 2).

The initial mean liver–somatic indexes ranged from 0.997 to 1.208; mean K values ranged from 1.90 to 2.10 and did not differ significantly between experimental trials (ANOVA, $P < 0.05$).

The green sunfish used in these trials exhibited some variation in nutritional value (Table 2). The caloric content exhibited the greatest variation between trials, but this had little effect on bass liver–somatic index values. When 800 cal/g wet green sunfish was used as the basis for recalculating the regression equations in Fig. 1, the given slope was changed <10%. In the 14.0°C trial the slope increased from 0.299 to 0.321, but in the 20.0°C trial the slope decreased from 0.322 to

0.302. No slope change was exhibited for the 28.0°C trial.

On a percentage basis, at increased feeding rates the relative moisture content of bass livers decreased slightly but not significantly at the 0.05 probability level. There was a significant increase in the percentage of glycogen and crude fat at higher feeding rates. The percentage of crude protein and ash decreased with higher feeding rates (Table 3).

Discussion

Under the physical conditions of the experiment the liver–somatic ratio was a sensitive index of daily food intake of the largemouth bass, while *K* was not nearly as sensitive. After a 14-day feeding trial the liver-somatic index was positively correlated with daily food intake of the bass at all three experimental temperatures. At 20.0 and 28.0°C there was no difficulty in differentiating between bass that had consumed 0 vs. 2% and 2 vs. 4% of their body weight per day, since the corresponding ranges of the liver–somatic index did not overlap. At 14.0°C the largemouth bass would not consistently consume more than 1% of their body weight in live green sunfish per day. In effect this reduced the range over which the liver–somatic index could be correlated with daily food intake. With the small sample size of five bass there was statistically no difference in the mean liver–somatic index at the feeding rates of 0 vs. 0.5 vs. 1% of body weight per day. Since at all temperatures used in this study the mean value of the liver–somatic index increased with increased daily ration, a large sample would likely result in a statistically significant difference in means.

The increase in the liver–somatic index with increased food consumption was due to a disproportionate increase in wet liver weight to body weight. Since water makes up approximately 75% of the liver's wet weight, additional water is primarily responsible for the absolute weight increase of the liver. On a percentage basis, at increased feeding rates there was a decrease in the water content and an increase in the fat content of the liver. This relationship has been found to be generally true for fish tissue (Iles and Wood 1965). The change is not more obvious because of the low fat content of bass liver. Hinton et al. (1972) also found that the largemouth bass liver has a low fat content and that glycogen is the primary storage product.

Within a given temperature trial, as the food consumed by the bass increased there was a linear increase in the liver–somatic index. Over a wider range of food consumption, or if more feeding rates were used, a curvilinear relationship may become evident.

At a given feeding rate there is a definite curvilinear reduction in the liver–somatic index as the temperature increases. This reduction may be due to increased maintenance requirement of the bass at the higher temperatures.

Under certain circumstances it may prove desirable to correlate the liver–somtic index with caloric intake. For example, since tadpoles have approximately one half as many calories per unit wet weight as fish, it is not likely that on a wet weight basis a 3% daily food intake of tadpoles would yield the same liver–somatic index as a 3% intake of green sunfish.

Due to the relatively small size of bass used in this study, the weight of the gonads had little effect on the liver–somatic index. However, with larger fish, especially those sampled in the spring, the weight of the gonad will make up a greater part of the body weight. Thus, the ovary weight may become at least as significant as the weight of the fish's stomach contents in causing variation within a sample.

The liver–somatic index, or any index of daily food intake, is of considerable potential use to the field biologist. With such an index the effect of drawdown, pollution, adding forage fish, partial poisoning, channelization, removing cover, artificial reefs, etc., could be monitored with respect to short-term changes in the daily food intake of the largemouth bass. These short-term changes could also be integrated over longer periods of time.

The results of this study are based on carefully controlled conditions. Experiments were conducted in aquaria at selected temperatures and feeding rates. The test fish were uniform in size and were all given the same type of food at regular intervals. More research including the effects of size, sex, maturity, seasons, and activity is needed and is currently being conducted to test the reliability of the liver–somatic index as an estimator of daily food consumption and an index of condition in wild fish populations. However, the method appears to have considerable potential. In its final form the fisheries manager would obtain bass samples, calculate the liver–somatic index, and use a table or family of curves to convert this information into daily food intake.

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Effects of Constant Temperatures and Diel Temperature Fluctuations on Specific Growth and Mortality Rates and Yield of Juvenile Rainbow Trout, *Salmo gairdneri*

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HOKANSON, K. E. F., C. F. KLEINER, AND T. W. THORSLUND. 1977. Effects of constant temperatures and diel temperature fluctuations on specific growth and mortality rates and yield of juvenile rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 34: 639–648.

Specific growth and mortality rates of juvenile rainbow trout (*Salmo gairdneri*) were determined for 50 days at seven constant temperatures between 8 and 22°C and six diel temperature fluctuations (sine curve of amplitude ±3.8 deg C about mean temperatures from 12 to 22°C). For constant temperature treatments the maximum specific growth rate of trout fed excess rations was 5.12%/day at 17.2°C. An average specific mortality rate of 0.35%/day was observed at the optimum temperature and lower. At temperatures in excess of the growth optimum, mortality rates were significantly higher during the first 20 days of this experiment than the last 30 days. The highest constant temperature at which specific growth and mortality rates became equal (initial biomass remained constant over 40 days) was 23°C. The upper incipient lethal temperature was 25.6°C for trout acclimated to 16°C. A yield model was developed to describe the effects of temperature on the living biomass over time and to facilitate comparison of treatment responses. When yield was plotted against mean temperature, the curve of response to fluctuating temperatures was shifted horizontally an average 1.5 deg C towards colder temperatures than the curve of response to constant temperature treatments. This response pattern to fluctuating treatments indicates that rainbow trout do not respond to mean temperature, but they acclimate to some value between the mean and maximum daily temperatures. These data are discussed in relation to establishment of criteria for summer maximum temperatures for fish.

Key words: constant temperature, fluctuating temperature, specific growth rate, specific mortality rate, yield, lethal temperature, zero net biomass, rainbow trout, thermal criteria

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Nous avons déterminé les taux de croissance et de mortalité spécifiques de jeunes truites arc-en-ciel (*Salmo gairdneri*) sur une période de 50 jours à sept températures constantes entre 8 et 22°C et avec six fluctuations nycthémérales de températures (courbe de sinus d'amplitude de ±3.8 deg C autour de températures moyennes de 12 à 22°C). A des traitements à température constante, le taux de croissance spécifique maximal de truites arc-en-ciel pourvues d'un excédent de nourriture est de 5.12%/jour à 17.2°C. On observe un taux de mortalité spécifique moyen de 0.35%/jour à la température optimale et à des températures plus basses. A des températures supérieures à la température optimale de croissance, les taux de mortalité sont nettement plus élevés pendant les 20 premiers jours de cette expérience que pendant les derniers 30 jours. La température constante la plus haute à laquelle les taux de croissance et de mortalité sont égaux (la biomasse initiale demeurant constante pendant plus de 40 jours) est de 23°C. La température supérieure du début des mortalités est de 25.6°C pour des truites acclimatées à 16°C. Un modèle de rendement a été élaboré pour décrire les effets de la température sur la biomasse vivante avec le temps et pour faciliter la comparaison des réponses au traitement. Si l'on porte le rendement contre la température moyenne, la courbe de réponse à des températures variables se déplace horizontalement sur une moyenne de 1.5 deg C vers des températures plus froides par rapport à la

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courbe de réponse à des traitements à températures constantes. Ce genre de réponse à des traitements variables indique que les truites arc-en-ciel ne réagissent pas à la température moyenne mais s'acclimatent à une valeur quelconque entre les températures quotidiennes moyenne et maximale. Nous analysons ces données en regard de l'établissement de critères touchant les températures maximales estivales pour les poissons.

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VARIOUS thermal criteria have been proposed to protect aquatic organisms (Ohio River Valley Water Sanitation Commission 1956; U.S. Department of the Interior 1968). Generally these criteria take the form of a maximum temperature that shall not be exceeded in summer months, and provide a limitation on the rate of temperature change or rise above ambient receiving water temperature. More recently the NAS/NAE (1972) recommended short-term maximum temperatures that shall not be exceeded to prevent heat death of aquatic organisms. A lower weekly average temperature (one-third the range between the optimum temperature and the ultimate upper incipient lethal temperature) was recommended to safeguard growth and activity of the desired species.

These estimates of optimum temperatures for growth, activity, and survival are estimates derived from constant temperature exposures. To apply these criteria to natural surface waters, an implicit assumption is made that the "average" temperature in constant and fluctuating temperature regimes gives the same response and common estimate of the optimum end point. The validity of this assumption must be tested since many fish experience large temperature changes in their natural environment. Needham and Jones (1959) reported diel fluctuations of 12 deg C in a trout stream. Brett (1971) described the diel vertical migrations of underyearling sockeye salmon, *Oncorhynchus nerka*, in Babine Lake, British Columbia. Young sockeye move into 17°C surface waters to feed at dusk and retreat to 5°C hypolimnetic waters at daylight. Spigarelli (1975) observed that Lake Michigan salmonids moved into thermal plumes; they did not acclimate to, or stay at, discharge temperatures for extended periods of time. These observations illustrate the prevalence of daily temperature changes in the fish's life history.

The study reported here was conducted at the Environmental Research Laboratory-Duluth as part of the continuing program to determine thermal requirements of important freshwater fishes. The purpose was to test the assumption that the average fluctuating temperature causes the same biological response as an equal constant temperature. The long-term effects of various constant temperatures and diel temperature fluctuations on specific growth and mortality rates of juvenile rainbow trout, *Salmo gairdneri*, are described. Observed growth and mortality rates are converted into a yield model to facilitate comparison of responses to both test conditions. The observed results are discussed in relation to establishment of thermal criteria for fish.

Materials and Methods

EXPERIMENTAL TANKS

Twenty-six 19-ℓ aquaria were used. A drain at the downstream end maintained water depth at 12.5 cm and 10 ℓ in each tank. Duplicate tanks were placed in a circulating water bath (57 × 40.5 × 15 cm deep) for each fluctuating temperature treatment. Fluorescent lighting controlled by a timer switch, adjusted weekly, followed the seasonal day-length cycle at Duluth, Minn. Light intensity at the water's surface among all tanks averaged 3.6 lm (range 0.5–10.0 lm).

WATER SUPPLY

Test water was obtained directly from Lake Superior (Hokanson et al. 1973). The water flowed through the following plumbing materials: polyvinyl chloride (PVC) pipes and valves into a stainless steel headbox, rigid plastic manifold, and stainless steel coils with Tygon® tubing connectors. Flow rate was controlled by glass capillary tubing, and aerated water was directed to the bottom of the aquarium by a standpipe. Flow rates were maintained at 287 ± 23 ml/min (mean ± 1 SD, range 176–332 ml/min). Dissolved oxygen concentrations were maintained at 91 ± 6% (mean ± 1 SD, range 68–107%) of air saturation as determined from 275 grab samples. Duplicate tanks were sampled alternately 3 times weekly. The pH varied from 7.2 to 7.7 in 55 samples.

TEMPERATURE CONTROL

Two systems controlled temperatures. The test water flowed through stainless steel heat exchangers immersed in the water bath for fluctuating temperature adjustment. Temperature was regulated by a controller that operated two solenoid valves and flow of hot or cold water into the bath. Temperature information was contained in a dual cam programmer with cam speed set for a 24-h revolution. Plexiglass cams were cut to achieve the desired diel temperature cycle (24-h periodicity). Nominal treatments were

programmed to follow a sine curve with amplitude of ± 4 deg C about the daily mean temperature. The minimum temperature was set at 0400 CDT, and the maximum temperature was set at 1600 CDT. Constant temperature control was achieved by thermostatic regulation of immersion heaters in a headbox which dispersed test water through a manifold system to experimental chambers.

Thermal treatments consisted of seven constant temperatures between 8 and 22°C and six diel temperature fluctuations with daily means between 12 and 22°C (Fig. 1). Multipoint thermographs were calibrated daily with a mercury thermometer to the nearest 0.1 deg C. The reported grand average temperature of each treatment was computed from daily means derived from hourly values. The maximum variation (± 2 SD, N = 50) of grand average constant temperatures was ± 0.6 deg C and for fluctuating temperatures ± 0.8 deg C. The maximum of fluctuating temperatures was controlled within ± 0.9 deg C and the minimum within ± 1.8 deg C.

Experimental Fish

Approximately 4800 rainbow trout eggs were obtained from two Lake Superior stock females. The eggs, fertilized May 23, 1972, were reared in a Heath incubator and held initially at 6–8°C. Eyed embryos were held at 9–12°C until hatching on July 5, 1972. Total hatch of eggs was 87%.

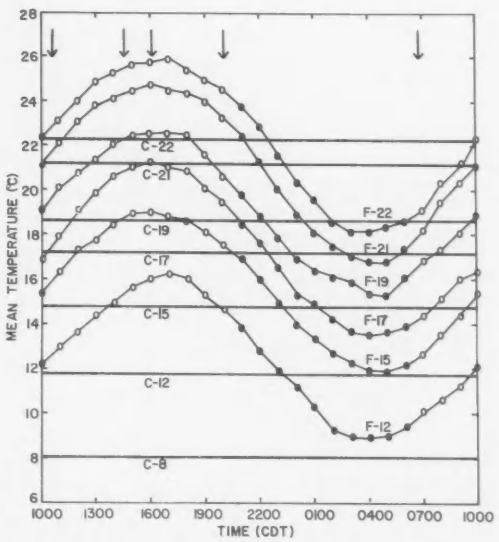

FIG. 1. Mean temperature plotted against time of day in hourly intervals for seven constant (C) and six diel fluctuating (F) treatments. Average amplitude of fluctuating treatments was ±3.8 deg C. ○, daylight hours and ●, hours of darkness for August 25. Arrows represent the median time of five ½-h feeding periods.

Alevins were transferred to two fiberglass tanks (150 × 27 × 35 cm deep) partially covered with black plastic. Each tank was provided with a water flow of 2–4 ℓ/min. Water temperature was increased gradually to 16°C when alevins commenced feeding on July 14. Trout were fed EWOS salmon and trout starter twice a day. Total survival of alevins was 89%.

Rainbow trout, acclimated to 16°C, were randomly distributed to each test tank between 1000 and 1315 CDT, August 1. Fifty trout were placed in each tank for a total of 100 per treatment. A random sample of an additional 50 trout averaged 30.2 mm (range 25.5–34.0 mm) total length and 245 mg (range 126–365 mg) wet weight. This common estimate of initial size applies to all treatment groups.

Growth

The trout were fed a varied diet consisting primarily of Glencoe Mills and Oregon Moist trout pellets. Fish were fed to satiation 5 times daily. Median feeding times were 0645, 1025, 1415, 1600, and 2000 CDT (Fig. 1).

Growth was followed for 50 days by placing a nine-celled sampling grid in each experimental tank every 10 days and selecting a random sample of about one-fifth the number of survivors (i.e. 10 or fewer fish). All sampled trout were preserved in 10% buffered formalin, blotted damp-dry, and wet weights were recorded to the nearest milligram.

Graphical inspection of the growth data showed that growth rates were constant over the 50-day period so that the weight at time t could be expressed as

$$W_t = W_o^{e^{g_j t}}$$

where g_j is the instantaneous growth rate for the jth experimental treatment group. However, since each treatment group started out with a common estimate of initial weight (W_o), treatments were not independent of one another. It is more efficient to recast the problem as one in multiple linear regression and force the regression lines through a common origin so that g_j could be estimated in the linearized form.

$$\ln W_t = \ln W_o + g_1 x_1 + g_2 x_2 + \ldots\ldots + g_k x_k$$

where $x_j = t$ if the observations come from the jth treatment group and $x_j = 0$ for all other treatment groups. The parameters $\ln W_o$, g_1, g_2, ... ,g_k and their standard errors were computed by using the BMDO3R multiple linear regression program described in Dixon (1970). Specific growth rates were calculated as $g_j \times 100$. Differences between regression coefficients were tested as suggested by Steel and Torrie (1960).

Mortality

The upper incipient lethal temperature of rainbow trout acclimated to 16°C was determined 10 days before start of the growth study to set the maximum

for thermal treatment. Subsamples of 10 trout were transferred abruptly into each experimental tank set at constant temperatures of 24.5, 25.4, and 26.3°C. They were not fed for 24 h before transfer or during this test. Percentage survival was recorded, and the 24- and 96-h median tolerance limits (TL50) were derived for duplicate tests.

Deaths of rainbow trout were recorded daily during the growth experiment. Daily instantaneous mortality rates (i) were derived (Ricker 1958) at 5–10-day intervals because mortality rates were not constant. Mortality rates were assumed to be constant over short time intervals so that i was estimated using the linearized equation

$$(L_n N_o - L_n N_t)/(t_t - t_o)$$

where N_t is the number of survivors at time t and N_o is the initial number of fish at time t_o. Specific mortality rates were calculated as $i \times 100$.

Yield

Yield is defined as the living biomass at any given instant equivalent to the concept of standing crop of field investigations, or the change in stock size (Ricker 1958). A yield model was developed because we sampled fish without replacement, which does not allow one to follow the living biomass as a function of thermal treatments. A hypothetical test population was created, which allowed the observance of changes in living biomass over time and facilitated comparisons of both constant and fluctuating temperature regimens by evaluating one response (yield) instead of two (constant growth rate and variable mortality rate). The yield (Y_t) at time t may be expressed as

$$Y_t = N_t \cdot W_t = N_o \cdot P_t \cdot W_o e^{gt}$$

where N_t, N_o, W_t, and W_o were previously defined, and P_t is the probability of surviving until time t. The value of P_t is estimated by taking the product of the observed proportion of survivors over each 5–10-day sampling interval. The fate of a hypothetical test population is described given an initial number (N_o = 10,000) of rainbow trout and a common estimate of average initial weight (W_o = 227 mg). Yield (kilograms) was estimated at 5–10-day intervals for each treatment from corresponding estimates of growth and mortality rates.

Thermal Requirements

Growth

The growth of rainbow trout from sample estimates and fitted regression lines is illustrated in Fig. 2 for two treatments. Mean sample weights did not always fall below the fitted regression line in the later sampling intervals, and deviation from regression showed no consistent trend that could be attributed to sampling error. Therefore, growth rates were assumed to be constant for all treatments in the 50-day test period.

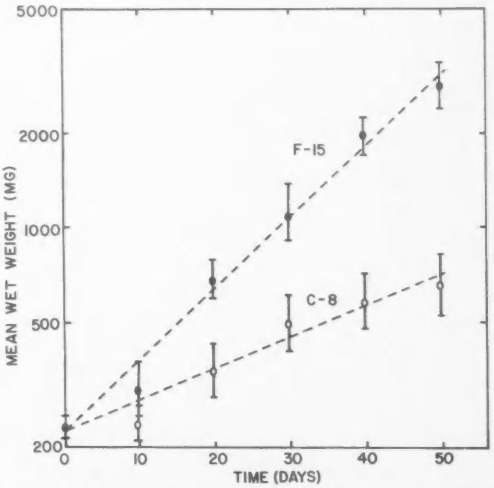

FIG. 2. Semilogarithmic plot of mean wet weights of rainbow trout (*Salmo gairdneri*) sampled at 10-day intervals for two treatments (C-8, constant 8°C; F-15, fluctuating temperature, mean 15°C). Duplicate samples from each treatment were similar; therefore, they were combined to provide a single estimate of mean weight. Vertical lines are 95% confidence limits of sample mean weights. Dashed lines are computed regressions for overall growth at each treatment. The common origin (W_o) for all treatments was 5.4237 (antilog 227 mg).

The data and computed specific growth rates for each treatment are presented in Table 1. The specific growth rates and corresponding 95% confidence limits are plotted against mean daily temperature in Fig. 3. Growth rates increased with increasing temperature, reaching a maximum of 5.12%/day at C-17 and 5.24%/day at F-15. The optimum range for growth for constant treatments was 17.2–18.6°C and for mean fluctuating treatments (amplitude ± 3.8 deg C), 15.5–17.3°C. Growth rates at higher temperatures declined with increasing temperature. Growth at the highest fluctuating treatment (F-22) was not significantly different from zero ($P < 0.10$, t-test). Although corresponding constant and fluctuating treatment means were not significantly different in the design of this study, the trends in treatment differences were consistent (Fig. 3). When the data were plotted the growth curve at fluctuating temperatures was generally shifted to the left of the growth curve at constant temperatures. Thus growth of rainbow trout appears to be accelerated at fluctuating treatments when the mean temperature is below the constant temperature optimum for growth and retarded by mean fluctuating temperatures above the constant temperature optimum.

TABLE 1. Specific growth rates of rainbow trout (*Salmo gairdneri*) for each treatment and tests of significant differences. The common initial size for all treatments was 245 mg, and the origin of the multiple linear regression was 5.4237 (antilog 227 mg).

Nominal[a] treatment	Mean temp (°C)	No. fish	Growth period (*days*)	Specific growth rate (%/*day*)	
				Slope	±SD
C-8	8.1	94	50	2.29	0.148
C-12	11.8	65[b]	50	3.66	0.172
C-15	14.8	86	50	4.36	0.152
C-17	17.2	85	50	5.12[c]	0.153
C-19	18.6	94	50	4.83[c]	0.148
C-21	21.2	61	50	4.49	0.169
C-22	22.3	37	50	3.94	0.194
F-12	12.5	86	50	4.23	0.154
F-15	15.5	86	50	5.24[c]	0.155
F-17	17.3	79	50	4.86[c]	0.164
F-19	19.1	81	50	4.58	0.157
F-21	21.0	18	40	4.48	0.274
F-22	22.2	5	7	−2.12[d]	2.642

[a]Constant (C) and fluctuating (F) treatments.

[b]One aquarium overflowed and 28 trout escaped on August 15, 1972.

[c]Slope not significantly different from optimum at F-15 ($P < 0.05$, *t*-test).

[d]Slope not significantly different from zero ($P < 0.10$, *t*-test).

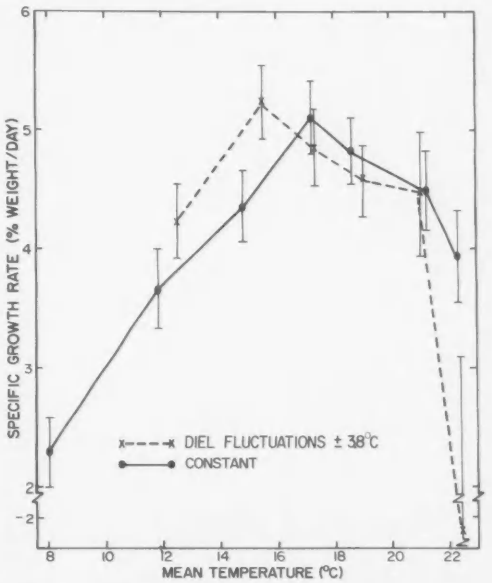

FIG. 3. Effect of constant temperatures and diel temperature fluctuations on specific growth rate of rainbow trout fed excess rations. Vertical lines are 95% confidence limits of the regression coefficient.

MORTALITY

The upper incipient lethal temperature (101 h) of the sample of rainbow trout acclimated to 16°C was 25.6°C. The mean 24-h TL50 was 25.7°C. All trout survived at 24.5°C for 101 h. The upper incipient lethal temperature was exceeded for 3 h daily only in the highest fluctuating treatment (F-22).

The average specific mortality rate was 0.36%/day (range 0–2.11%/day) at temperatures within the optimum range and lower (Table 2). Death occurred primarily among smaller individuals and apparently was related to stress from social interactions. Only at treatments in excess of the physiological optimum did we observe increased mortality rates during the first 20 days of this experiment. Mean lengths of dead fish at higher temperatures were similar to those of surviving fish. Mortality rates at temperatures above the respective optima were not constant, but generally reached a maximum in the first 10 days of the study. Thereafter mortality rates declined reaching an average for all treatments of 0.18%/day (range 0–1.25%/day) after 20 days. In the highest fluctuating treatment (F-22), mortality rate was high, 42.8%/day, during the first 7 days; consequently the fish did not survive to the end of the first sampling interval. Mortality rates generally were higher among the fluctuating treatment groups than among the corresponding constant treatment groups for the same time period. Apparently mortality rates of rainbow trout at elevated temperatures were also influenced by the thermal experience above the mean daily temperature.

YIELD

Based on the yield model, yield of 10,000 rainbow trout (average initial wet weight 227 mg) fed an excess daily ration was computed and plotted against time for three treatments (Fig. 4). The population maintenance level represents the initial weight of 2.27 kg for this hypothetical population and is the expected yield whenever the specific growth and mortality rates are equal over a given time interval. The response to C-19 represents a fast growing population with good survival rates. After 40 days, 9429 fish survive with an average wet weight of 1562 mg. The response to C-8 represents a slow-growing population with good survival rates. After 40 days, 9468 fish survive with an average wet weight of 567 mg. The yield at F-21 declined to a low of 0.69 kg after 10 days because the specific mortality rate exceeds the growth rate during this time interval. The yield increased to 1.96 kg after 40 days when

TABLE 2. Specific mortality rate (percent per day) of rainbow trout for different thermal treatments and time intervals.

Nominal[a] treatment	Mean temp (°C)	Time interval (*days*)						
		0–5	5–10	10–15	15–20	20–30	30–40	40–50
C-8	8.1	0.20	0.20	0	0	0.34	0	0.51
C-12	11.8	0	0.21	0	0.41	0	0	1.25
C-15	14.8	0	0.23	0.30	0	0	0	0
C-17	17.2	0.66	0.45	0.60	0.31	0	0	0
C-19	18.6	0.61	0	0.21	0	0.18	0	0
C-21	21.2	2.49	4.58	1.19	0	0.54	0	0
C-22	22.3	3.61	13.62	1.33	0.70	0	0	0.57
F-12	12.5	0	0.21	0.56	0.57	0.20	0.31	0
F-15	15.5	0	1.06	0.85	0	0.19	0.29	0
F-17	17.3	0.63	0.66	2.11	0.32	0	0	0.65
F-19	19.1	0.84	1.57	0.60	0	0.20	0.31	0
F-21	21.0	11.49	21.29	4.46	0	0.74	0	—
F-22	22.2	46.05	34.66	—	—	—	—	—

[a]Constant (C) and fluctuating (F) treatments.

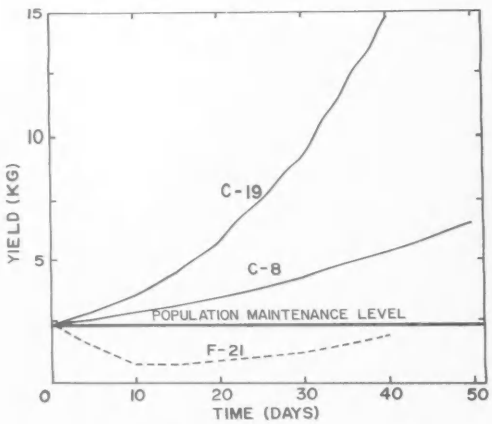

FIG. 4. Yield of 10,000 rainbow trout (initial wet weight 227 mg) at 10-day intervals for three treatments. The population maintenance level represents the initial weight of the test population and the expected yield when the specific growth and mortality rates are equal.

the mortality rate declined. This population consisted of 1442 fish with an average wet weight of 1360 mg.

The yield of this hypothetical rainbow trout population at different time periods and thermal treatments is shown in Table 3. To compare responses to constant and fluctuating treatments, the 40-day yield is plotted against mean daily temperature in Fig. 5. A maximum yield of 15.87 kg was produced at C-17, and 15.96 kg at F-15 after 40 days. Yields declined at lower temperatures because of reduced growth rates and at higher temperatures because of reduced growth rates and increased mortality rates. When the specific growth rate equals the specific mortality rate the population experiences no change in weight (zero net biomass gain). The maximum temperature at which a population of rainbow trout can maintain its initial weight for 40 days is a constant temperature of 23°C and a fluctuating mean temperature (± 3.8 deg C amplitude) of 21°C. In graphic presentation the yield in response to fluctuating temperatures was generally shifted towards colder temperatures than the curve of response to constant temperatures. A graphical estimate of the average shift was 1.5 deg C which was constant for all time intervals.

Implications for Thermal Criteria

Development of water quality criteria involves the identification of graphical end points that have particular physiological and ecological significance (Brett 1971; NAS/NAE 1972). Criteria are not designed to produce maximum growth of fish, but to protect a balanced and indigenous fauna of direct importance to man. The physiological optimum and upper incipient lethal temperature of a species is used directly in derivation of summer-limiting temperatures for aquatic life. Measures of physiological performance and activity in constant temperature studies point to an optimum of 16–18°C for rainbow trout (Lichtenheld 1966; Lavrovsky 1968; McCauley and Pond 1971; Dickson and Kramer 1971). The upper incipient lethal temperature (25.6°C) of this stock of rainbow trout was almost identical to those reported for other stocks of this species (Bidgood

TABLE 3. Yield (kilograms) of 10,000 rainbow trout (initial wet weight 227 mg) for different thermal treatments and time periods.

Nominal[a] treatment	Mean temp (°C)	Time (*days*)						
		5	10	15	20	30	40	50
C-8	8.1	2.52	2.79	3.13	3.51	4.27	5.37	6.42
C-12	11.8	2.72	3.24	3.89	4.57	6.59	9.51	12.10
C-15	14.8	2.82	3.47	4.24	5.28	8.16	12.62	19.50
C-17	17.2	2.83	3.58	4.49	5.71	9.62	15.87	26.48
C-19	18.6	2.80	3.56	4.49	5.71	9.09	14.73	23.87
C-21	21.2	2.51	2.49	2.94	3.68	5.46	8.56	13.41
C-22	22.3	2.31	1.42	1.62	1.90	2.83	4.19	5.87
F-12	12.5	2.80	3.42	4.11	4.94	7.39	10.94	16.70
F-15	15.5	2.95	3.63	4.52	5.87	9.73	15.96	26.94
F-17	17.3	2.80	3.45	3.96	4.97	8.08	13.13	20.01
F-19	19.1	2.73	3.18	3.88	4.87	7.55	11.58	18.30
F-21	21.0	1.60	0.69	0.69	0.86	1.25	1.96	—
F-22	22.2	0.20	0.10	—	—	—	—	—

[a]Constant (C) and fluctuating (F) treatments.

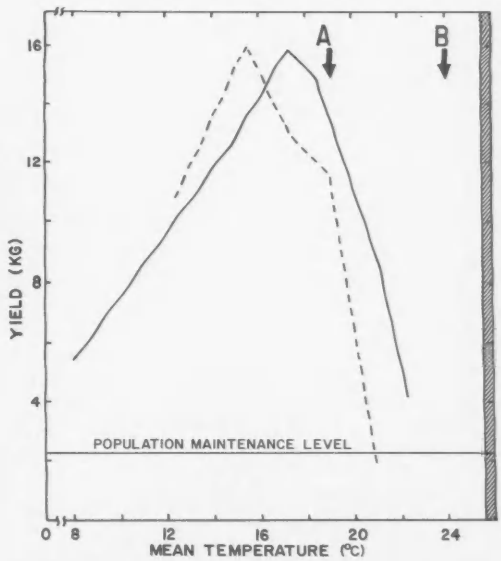

FIG. 5. Effect of constant temperatures (solid line) and diel temperature fluctuations (dashed line) on the 40-day yield of 10,000 rainbow trout (initial mean weight 227 mg). The shaded area represents the zone of resistance in excess of the upper incipient lethal temperature. Recommended water-temperature criteria for rainbow trout are defined by arrows (National Academy of Sciences 1972): (A) maximum weekly average temperature and (B) short-term maximum for survival.

and Berst 1969; Alabaster 1964). Mortality rates of rainbow trout fed to satiation increased significantly above the physiological optimum temperature. Other authors have also reported increased mortality rates in growth experiments at apparently sublethal temperatures (Strawn 1961; Allen and Strawn 1968; Brett et al. 1969). Mortality rates of rainbow trout were higher than one might expect as 100% survived temperatures within 1 deg C of their upper incipient lethal temperature in short-term tests where fish were not fed 24 h before or during exposure to lethal temperatures.

Temperature criteria recommended for rainbow trout waters by the NAS/NAE (1972) are indicated by arrows in Fig. 5. The maximum weekly average temperature (19°C, point A) is set at one-third of the range between the optimum temperature reported in earlier studies and the ultimate upper incipient lethal temperature. The maximum temperature (24°C, point B) for short-term survival is based on a 24-h TL50 minus 2 deg C for fish acclimated to the mean temperature (19°C). This 2 deg C "safety factor" was estimated by Coutant (1973). Care should be exercised in the application of a formula derived from short-term entrainment studies to the long-term effects of a modified temperature regime over a wider area of the receiving water. The harvestable yield depends on environmental effects on population growth and mortality over time. The maximum temperature at which a rainbow trout population can be expected to maintain its weight for 40 days was a constant temperature of 23°C and a fluctuating mean temperature (± 3.8 deg C) of 21°C. The temperature range from 21 to 23°C has also been reported by various authors as representing the upper limits of rainbow trout distribution (Burton and Odum 1945; Horak and Tanner 1964; Pierce 1966; McCauley and Pond 1971). Apparently temperatures selected by rain-

bow trout in their natural habitat are below the upper zero net gain value whereby a sustained yield will occur. The criteria of the National Academy of Sciences applied to our test conditions would allow a 13% reduction from maximum 40-day yield for constant temperature exposures and a 27% reduction from maximum yield for fluctuating temperature regimes.

Numerous studies and observations give conflicting views on effects of fluctuating temperatures. The limitation on rate of temperature change in thermal criteria is based, in part, on the knowledge that death of aquatic organisms can occur if they are transferred from one water temperature to another too quickly without suitable acclimation. Most fish species, however, can survive sudden temperature changes up to 15–18 deg C within their respective thermal tolerance limits (Hart 1947, 1952). Sublethal temperature changes have often been labeled stressful by physiologists who have observed changes in tissue function (Hughes and Roberts 1970; Wedemeyer 1973; Heinicke and Houston 1965; Coutant 1973). It is not clear whether the measured changes from short-term experiments were deleterious to fish or were merely adaptive changes in the process of thermal acclimation. Brett (1971) postulated that the diel vertical migrations of salmon to colder waters after feeding in warm surface waters may be an energy conservation mechanism in food-limited environments. In the present study, diel temperature fluctuations within the range normally selected by rainbow trout (i.e. physiological optimum and lower) were beneficial to growth. Fluctuations at mean temperatures above the physiological optimum, however, are more harmful than currently estimated from constant temperature exposures in most laboratory studies.

The observed response to fluctuating temperatures can be attributed to the differential rates of thermal acclimation to high and low temperatures. Body temperatures of alewives, *Alosa pseudoharengus*, equilibrated faster to increasing than decreasing temperature changes (Spigarelli et al. 1974). Brett (1946) acclimated fish to increasing and decreasing temperatures for various time periods. By noting changes in their lower and upper lethal temperatures, he found that they acclimated faster to higher than to lower temperatures. Heath (1963) acclimated cutthroat trout, *Salmo clarki*, to constant and cycled temperatures (10–20°C). Maximum tolerance to high temperature was observed on a 24-h acclimation cycle, and the lethal temperature of cycled trout suggests that they acclimate to the peak (20°C) rather than to the mean temperature. Fish acclimated to diel temperature fluctuations compensate for both warm and cold temperatures simultaneously and have an increased range of thermal tolerance (Feldmeth et al. 1974). Amargosa pupfish, *Cyprinodon nevadensis*, acclimated to a diel temperature cycle from 15 to 35°C had critical thermal maxima equal to those acclimated to constant 35°C and had critical thermal minima equal to those acclimated to constant 25°C (the mean of the cycle). The critical thermal minimum was changed more slowly reaching its lowest point after 22 days of acclimation compared to the maximum which was reached in less than 7 days.

The convention of plotting biological response against mean temperature has not been justified by this study. The observed shift (1.5 deg C) in response between constant and fluctuating treatments (± 3.8 deg C) is consistent between all treatments and time intervals although this difference may not be statistically different in the design of this study. The amount of shift in response to fluctuating treatments is a direct function of the amplitude of the diel cycle (Environmental Research Laboratory-Duluth, unpublished data). These data trends indicate that a shift in response greater than 1 deg C may occur when diel temperature variations exceed ± 2 deg C. A method to correct this difference in response is desirable before we can validly apply results from simplified laboratory experiments conducted at constant temperatures to field conditions with large diel temperature changes or habitat gradients. These results indicate that instantaneous response may lag behind the instantaneous temperature experience and that temperatures above the mean exert greater influence than below. As a hypothesis we will assume that under a fluctuating temperature regime an organism will acclimate to some function of its temperature exposure over time (i.e. between the median or 50th percentile and the maximum or 100th percentile of the fluctuating temperature duration). This hypothesis should be tested for different species and temperature cycle periodicities and amplitudes before one can derive the specific mathematical function to predict fish response to irregular temperature cycles.

Derivation of temperature standards must take into account the magnitude of temperature variation of the natural environment to which these criteria apply. From the foregoing discussion, the mean weekly temperature should be conservatively retracted towards the optimum end point derived from constant temperature studies (i.e. 17°C for rainbow trout) to achieve the desired degree of protection recommended by the NAS/

NAE (1972) where diel temperature variations exceed ± 2 deg C. The temperature at which the specific growth and mortality rates become equal in constant temperature experiments (i.e. upper zero net biomass gain for rainbow trout is 23°C) should set the maximum thermal limit at any time or place where a sustained yield is desired. These end points were estimated from maximum ration studies. These limits should be further retracted towards colder temperatures to safeguard natural populations receiving less than full rations (Brett et al. 1969; Fry 1972). The specific mathematical function that describes acclimation to fluctuating treatments should be derived in future studies so that results of constant temperature studies can be universally applied in setting water temperature standards.

Acknowledgments

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Precipitation Loading of Acid and Heavy Metals to a Small Acid Lake near Sudbury, Ontario

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BEAMISH, R. J., AND J. C. VAN LOON. 1977. Precipitation loading of acid and heavy metals to a small acid lake near Sudbury, Ontario. J. Fish. Res. Board Can. 34: 649–658.

The pH of Lumsden Lake was closely related to the measured amount of acid entering the lake from bulk precipitation. In 1972 it was estimated that an excess of 2135 kg of H_2SO_4 was added to the lake from the atmosphere. The predicted annual change in lake pH was from 5.2 to 4.8 and the actual measured change was from 5.2 to 4.7. In 1973 an estimated excess of 1271 kg of H_2SO_4 was added to the lake from the atmosphere. The predicted annual pH change was from 4.8 to 4.7 and there was no net change in the lake pH in 1973.

Lumsden Lake also had high concentrations of sulfate, manganese, zinc, and nickel in comparison with remote or "unpolluted" lakes. High concentrations of manganese and possibly some zinc in the lake appeared to result from increased mobilization from the lake sediments or watershed or both as the pH decreased. Atmospheric fallout also contributed substantial quantities of nickel and copper to the watershed, some of which appeared to be retained in the lake water. The high concentrations of lead in the precipitation were not retained in the lake.

BEAMISH, R. J., AND J. C. VAN LOON. 1977. Precipitation loading of acid and heavy metals to a small acid lake near Sudbury, Ontario. J. Fish. Res. Board Can. 34: 649–658.

Le pH du lac Lumsden est étroitement lié à la quantité d'acide mesurée qui entre dans le lac avec la précipitation brute. On estime qu'en 1972 plus de 2 135 kg de H_2SO_4 ont été ajoutés au lac en provenance de l'atmosphère. Le changement annuel prédit dans le pH du lac était de 5.2 à 4.8, alors que le changement mesuré réel était de 5.2 à 4.7. En 1973 on estime à plus de 1 271 kg la quantité de H_2SO_4 ajoutée au lac en provenance de l'atmosphère. Le changement de pH prédit pour l'année était de 4.8 à 4.7, et il n'y eut pas de changement net de pH dans le lac en 1973.

Le lac Lumsden contient en outre de fortes concentrations de sulfate, manganèse, zinc et nickel comparativement aux lacs éloignés ou « non pollués ». Les hautes teneurs en manganèse et possiblement une certaine quantité de zinc dans le lac semblent résulter d'une mobilisation accrue depuis les sédiments du lac, le bassin hydrographique ou les deux, à mesure que le pH diminue. Les retombées atmosphériques fournissent également au bassin hydrographique des quantités substantielles de nickel et de cuivre, dont une partie semble être retenue dans l'eau de lac. Les hautes teneurs en plomb dans les précipitations ne sont pas retenues dans le lac.

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MANY lakes in the vicinity of Sudbury, Ont., have been found to be extremely acidic (Beamish and Harvey 1972) and earlier studies have concluded that precipitation was the most probable source of acid to these lakes. Because no measurements were available that directly related acid in the precipitation to changes in acid content of lakes, in 1972, a 2-yr study was undertaken to examine precipitation-induced changes in acid and heavy metal content in one of these acid lakes.

Lumsden Lake was chosen as the study site because it was one of the more acidic lakes, was small with a small drainage area, and was one of the more accessible (by foot) of the acidified lakes (see Beamish and Harvey 1972 for diagram of Lumsden and associated lakes). Extensive fisheries studies had also been conducted in Lumsden Lake and the disappearance of almost all species has been documented (Beamish and Harvey 1972; Beamish 1974).

Materials and Methods

Lake inlet and outlet water samples were collected at least bimonthly over the 2-yr period from 1972 to

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1973. Precipitation collectors for chemical analysis samples were set up on top of a small elevation of exposed bedrock. Precipitation was collected continually and also retrieved at least bimonthly. However, some samples were destroyed by animals. Clear polyethylene funnels supported by a 120-cm high wood frame drained into clear polyethylene bottles. Funnels with a small inside stem diameter were chosen to reduce the amount of evaporation. Collection bottles were protected from evaporation and spillage by a casing of gravel held in place by a wood frame. Distilled-water-rinsed clear polyethylene plastic bags replaced the funnels in the wooden frame for the collection of snow. Fallen snow was collected with an aluminum scoop, and a vertical section of snow was placed into acid-washed polyethylene containers and acid- and nonacid-washed clear polyethylene plastic bags. "Event" rains or snows were collected by placing the appropriate cleaned container in an open field after the event started and removing it before the end of the event.

A VanDorn water sampler was used to collect lake water in clean nonacid-washed polyethylene bottles for all determinations other than heavy metals. Acid-washed distilled-water-rinsed polyethylene bottles were used to store samples for heavy metal analysis. Inlet and outlet water samples were obtained by hand holding an empty bottle in the current such that one's hand was downstream of the bottle opening. The pH was determined and checked in a field laboratory within several hours of sampling using two pH meters. Concentrations of substances other than heavy metals were determined within the acceptable storage times according to the procedures currently in use at the Freshwater Institute (Stainton et al. 1974). Concentrations of heavy metals were determined by an ammonium pyrothidine dithiocarbonate–methyl isobutyl ketone procedure similar to the method described by Brooks et al. (1967) and VanLoon and Beamish (1977). The concentrations of these metals were determined using Perkin Elmer Model 303 and instrumention laboratories Model 153 atomic absorption spectrophotometers. Standard reference samples of trace metals supplied by the Environmental Protection Agency, Cincinnati, Ohio, were also analyzed to evaluate the accuracy of the determinations. In an earlier study it was found that except for iron the concentrations of heavy metals in samples from these oligotrophic lakes were not affected by preserving the sample by freezing (Beamish 1974). Thus, samples that could not be analyzed for heavy metal concentrations within several days were frozen prior to analysis. As a consequence of freezing, the analyzed concentrations of iron may be low.

Lumsden Lake was estimated to have a volume of 240×10^4 m^3, a surface area of 21 ha, a maximum depth of 22 m, and a mean depth of 7 m. Drainage area was estimated from topographical maps and by visual observation to be 98 ha. The lake surface and surfaces that drained directly into the lake were considered to represent the drainage area. Areas draining into the inlet were not added to the drainage area.

Outflow volume was determined initially using a current meter; however, after May 1972 the amount of water passing through a "V" notch was monitored with a continuous water-level recorder and converted to volume.

The total annual amount of precipitation was estimated using the values measured by the Department of Transport records of meteorological observations in Canada at Sudbury Airport. Although this site was approximately 60 km N.E. of the lake, it provided a better estimate of total precipitation because in situ gauges were periodically overturned by animals.

The outlet volume in 1972 was 269×10^4 m^3 and 297×10^4 m^3 in 1973. Total annual precipitation in 1972 was 95×10^4 m^3 and 81×10^4 m^3 in 1973. The larger outlet volume in 1973, despite the reduced amount of precipitation, resulted from breakage of a beaver dam blocking a lake several lakes upstream of Lumsden Lake. The flushing period of the lake was estimated to be 11 mo in 1972 and 10 mo in 1973. Inlet volume was calculated as the outlet volume minus precipitation volume plus one third of precipitation volume to account for evapotranspiration (Hutchinson 1957, p. 524–525). The inlet volumes were calculated monthly and summed to be 241×10^4 m^3 in 1972 and 249×10^4 m^3 in 1973.

The total hydrogen ion content of the lake was calculated using an average annual pH calculated from the monthly or biomonthly samples (Table 1). Monthly pH values were used to calculate total input of hydrogen ion to the lake from the inlet and precipitation and that being lost via the outlet. If a monthly pH value was missing, the pH of the proceding monthly sample was used. Hydrogen ion concentrations were assumed to be equivalent to hydrogen ion activity and the result of sulfuric acid additions. Hence, weights or concentrations of sulfuric acid are used when considering the effects of precipitation on lake pH. To change the pH of the lake, acid must be added to the lake either from precipitation or from inlet in concentrations higher or lower than already present. In theory, a base or buffer would perform the same function. However, additions of buffer were not important, as the precipitation and inlet waters were strongly acidic.

The net weight of acid added annually to the lake in excess of the January concentration was computed using the following formula: excess acid = (mean inlet concentration − January lake concentration) inlet volume + (mean precipitation concentration − January lake concentration) precipitation volume − (mean outlet concentration − January lake concentration) outlet volume.

The expected change in the pH of Lumsden Lake to acid loadings was estimated using a titration curve produced prior to the study by adding 0.01 N HCl to a 200-ml sample of Lumsden Lake water (Fig. 1). The amount of acid required to acidify the volume of water in the lake plus the volume added to the lake each year was determined using this titration curve. We compared the response of Lumsden Lake to additions of acid with other lakes from the Experimental Lakes Area (ELA) and a less acidic lake (George Lake) in the vicinity of Lumsden Lake (Fig. 1). In the pH range 5.2–4.8 the George Lake curve had the steepest slope, thus requiring the least acid

TABLE 1. pH (H^+) concentrations from Lumsden Lake and precipitation. A dash indicates no data available.

	Inlet	Lake	Outlet	Precipitation		Surface snow
				Station	Event	
			1972			
Mean ± (2 SE)	5.0 (.22)	4.8 (.14)	4.8 (.14)	4.3 (.1)	4.3 (.46)	4.5 (.12)
Range	4.7–5.5	4.7–5.3	4.7–5.2	4.1–4.4	3.7–4.8	4.0–4.7
No. samples	7	8	7	8	4	12
Total annual wt H^+ (*kg*)	30.8	48.5	38.5	54.8	—	—
			1973			
Mean ± (2 SE)	4.8 (.06)	4.8 (.04)	4.8 (.08)	4.5 (.3)[a]	4.4 (.22)	4.3 (.10)[b]
Range	4.7–5.0	4.8–4.9	4.7–5.0	4.2–5.3	4.0–5.5	4.0–4.4
No. samples	8	8	8	9	13	7
Total annual wt H^+ (*kg*)	41.7	38.4	41.6	32.6	—	—

[a]Significantly higher than 1972 ($P \leq 0.05$).
[b]Significantly lower than 1972 ($P \leq 0.05$).

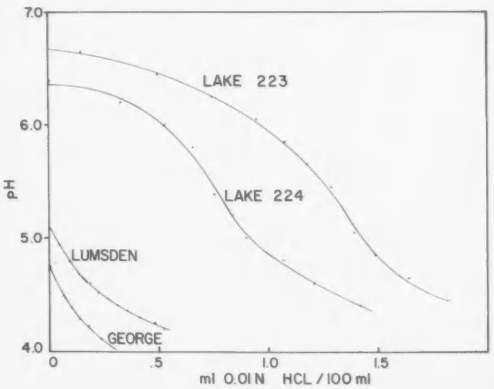

FIG. 1. Lake titration curves for two ELA and two Sudbury area Lakes.

to acidify an equivalent volume of water. The Lumsden Lake curve had the second steepest slope in this range followed by the ELA lakes, Lake 222 and Lake 224. The Lumsden Lake titration curve (Fig. 1) was used to predict the effect of acid additions to Lumsden Lake; however, other curves have been retained in this report as an example of differences among lakes. All lakes had low concentrations of major ions (Table 2).

One attempt was made to estimate the importance of accumulations of acid and heavy metals on surfaces between precipitation events. This study was conducted at a location 2 km N.W. of Lumsden Lake and while the chemical concentrations measured at this site could not be directly related to the chemistry of samples collected at Lumsden Lake, we considered that conclusions concerning the importance of accumulations of substances between precipitation events were applicable to the Lumsden Lake study. After a 10-day dry period, rain was collected early in an event at the same time run-off was being sampled. Runoff was sampled by holding a nonacid-washed container under a rock shelf so that water trickled into the container without the bottle touching the rock surface.

TABLE 2. Major ion concentrations (milligrams per litre) for two ELA and two Sudbury area lakes.

	Lake 224	Lake 223	Lumsden	George
Na	.7	.9	.4	.8
K	.3	.3	.3	.5
Ca	1.4	2.3	2.2	3.2
Mg	.5	.6	.6	1.0
Surface area (*ha*)	13.0	24.3	21	182

To assess the importance of concentrations found in the Lumsden Lake study, samples from lakes and precipitation from areas remote from industrialization were collected, analyzed, and compared with the Lumsden data. Surface snow samples were collected from a remote area in northern Manitoba. Lake water was analyzed (Beamish et al. 1976) from lakes situated in the Experimental Lakes Area (ELA) in northwestern Ontario, near the Ontario–Manitoba border. The ELA lakes (Table 3) have been considered to be "unpolluted". While this may not be true, the samples probably are only very slightly altered by human wastes. The 109 lakes sampled are remote from large industrialized areas, but Barica and Armstrong (1971) have shown that, occasionally, significant amounts of substances are deposited in the area from the atmosphere.

TABLE 3. Reference concentrations for precipitation and lake water. ND = not detected.

Constituent	"Unpolluted" snow (March 1972)	ELA lakes (109) (September 1973)	Lumsden Lake avg (1972 + 1973)
pH	6.3	6.5	4.8
SO_4 (*mg/ℓ*)	.2	4	10.7
Cl (*mg/ℓ*)	.4	.8	.9
Na (*mg/ℓ*)	.1	.9	.5
K (*mg/ℓ*)	ND	.3	.3
Mg (*mg/ℓ*)	ND	.6	.6
Ca (*mg/ℓ*)	.1	2	2.2
Cond (*μmhos/cm*)	<10	19	32
TDS (*mg/ℓ*)	—	—	18.3
TSS (*mg/ℓ*)	<3	2	1.0
Fe (μg/ℓ)	<2	81	35
Cu (μg/ℓ)	<1	2	4
Zn (μg/ℓ)	<1	<1	33
Pb (μg/ℓ)	<1	<1	2
Ni (μg/ℓ)	<2	<3	8
Cd (μg/ℓ)	—	<.1	.4
Mn (μg/ℓ)	<1	3	257
Alk ($CaCO_3$)(*mg/ℓ*)		10	<.5

Results

In 1972 the mean annual concentration of acid entering from the inlet converted from total weights (Table 2) to concentrations was 6.12×10^{-4} kg H_2SO_4/m^3 and the outlet concentration was a slightly higher 6.88 kg H_2SO_4/m^3. Since the lake concentration was 3.07×10^{-4} kg H_2SO_4/m^3 at the start of the study, the additional acid in the outlet water must have been added directly into the lake from precipitation or indirectly from runoff from the surrounding watershed. (Dry or aerosol fallout and wet fallout were collected by the bulk precipitation samplers and the term precipitation as used in this report refers to both types of fallout. Event precipitation samples contain only wet fallout). Precipitation contributed a mean concentration of 27.81 kg H_2SO_4/m^3 to the lake during 1972. It was calculated that 2050 km H_2SO_4 was added to the lake and volume of water passing into the lake during 1972, in excess of the January lake concentration. A weighted titration curve (Fig. 2) was calculated for the annual volume of water in the lake and the total volume of water passing into the lake. Using the curve calculated for 1972, it was estimated that the pH change would be from 5.2 to 4.8. The actual measured change was from 5.2 to 4.7.

In 1973 the mean annual concentration of acid added to the lake from the inlet was 8.04×10^{-4} kg H_2SO_4/m^3. The January lake concentration was 7.68×10^{-4} kg H_2SO_4/m^3 and the mean annual outlet concentration was 6.73×10^{-4} kg H_2SO_4/m^3. Precipitation in 1973 contained an average of 19.41×10^{-4} kg H_2SO_4 and contained significantly ($P \leq 0.05$) less acid than in 1972. The calculated excess acid added to the lake in 1973 amounted to 1314 kg H_2SO_4. Using the titration curve for 1973 that has been adjusted for the volume of water in and passing into the lake, it was estimated that the pH would change from approximately 4.8 to 4.7. The January 1973 and the December 1973 lake pH values were both 4.8; thus, there was no net change in pH in 1973. These pH values were identical to the 1973 mean

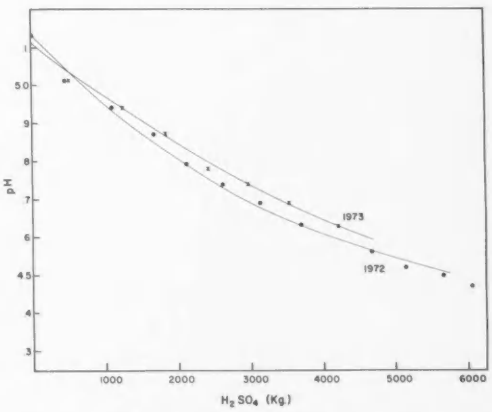

FIG. 2. Computed weight of sulfuric acid that must be added to total volume of water in and passing into Lumsden Lake to produce a change in pH.

TABLE 4. Ionic balance of precipitation and lake water. NA = not applicable.

	Precipitation (mEq/ℓ) stations		Lake (mEq/ℓ) stations	
	1972	1973	1972	1973
Cation				
Ca	.025	.034	.117	.098
Mg	.007	.003	.053	.053
Na	.012	.013	.019	.020
K	.003	.003	.007	.007
NH_4	—	—	NA	NA
H^+	.051	.032	.020	.016
Mn	NA	NA	.009	.009
Total	.098	.085	.225	.203
Anion				
SO_4	.115	.094	.223	.223
Cl	.020	.020	.031	.020
HCO_3	NA	NA	< .01	< .01
Total	.135	.114	.254	.243

annual concentrations found for the inlet, lake and outlet (Table 1).

Precipitation during 1973 was less acidic than in 1972 and reduced in volume. The mean pH of 4.5 at the precipitation stations in 1973 was significantly higher than the 1972 mean of 4.3 (Table 1). In contrast, the surface snow samples collected in 1973 were significantly more acidic than the 1972 samples ($P \leqslant 0.05$). Such differences emphasize that the pH of precipitation can be extremely variable. The mean pH of precipitation events and surface snows were similar to the mean monthly pH obtained from the precipitation collectors. Event samples were not available in sufficient quantity throughout the year to allow quantitative comparisons between precipitation station readings to be made. The conductivity values of event precipitation and precipitation station samples were similar, but there was some variation in total dissolved and suspended solids, indicating that the precipitation collectors probably were not contaminated except perhaps by organics such as pollen. The mean annual concentrations of cations and anions from the precipitation collectors did not balance (Table 4). A reasonable balance of anions and cations was achieved for the mean annual concentrations from lake water (Table 4); thus, the poor balance from the precipitation collectors probably was not a result of analytical errors. It is known that ammonium concentrations are high in polluted precipitation (Likens et al. 1976) ranging from 0.01 to 0.03 mEq/ℓ. Ammonium was not measured in this study and it is probable that much of the difference between anions and cations would be accounted for by this cation.

Large changes in vertical pH profiles were not observed in this study (Fig. 3) or in other studies in the same area (Beamish 1974) possibly because, in part, major loadings of acid were greatly reduced during the periods of reduced rainfall in the summer months when the lake was stratified.

HEAVY METALS

There was no significant change from 1972 to 1973 (t-test, $P \leqslant 0.05$) in the mean annual concentrations of heavy metals from any of the locations listed in Table 5. When concentrations of zinc, nickel, and manganese that were measured from Lumsden Lake and three other lakes during the study were compared with pH, the functions for zinc and nickel were weakly linear [$r = 0.34$ (Zn) and $r = 0.47$ (Ni)] but significant, indicating waters of lower pH contained higher concentrations of these metals. When only the Lumsden Lake samples were compared, zinc was the only heavy metal that could be shown to be significantly related to changes in pH ($P = 0.05$, $r = 0.61$). No relationship between differences in pH and manganese concentrations could be demonstrated for samples collected during this 2-yr period.

Manganese concentrations were extremely high (compared to ELA lakes) in the inlet, lake, and outlet (Tables 3, 5). These concentrations were significantly higher ($P \leqslant 0.01$) than the manganese from the precipitation station samples indicating that precipitation could not be considered to be an important supplier of manganese to the lake.

Concentrations of copper were high in precipitation and especially in snow (Tables 3, 5). Concentrations greater than 20 μg/ℓ were not uncommon in snow samples. In 1972 and 1973 concentrations of copper in the inlet, lake, and

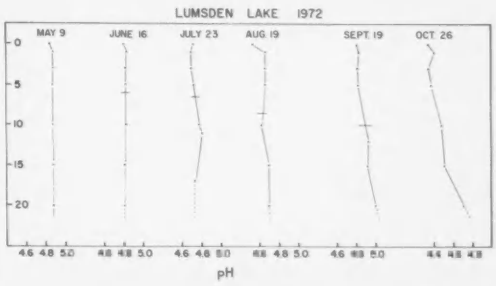

FIG. 3. Vertical pH, Lumsden Lake, 1972.

TABLE 5. Mean (±2 SE) heavy metal concentrations (micrograms per litre).

	Inlet	Lake	Outlet	Precipitation		Surface snow
				Station	Event	
			1972			
Zn	27.6 (3.8)	36.4 (14.6)	38.4 (12.6)	22 (12)	25 (30)	10.2 (6.4)
Cu	6 (2.6)	5 (5.6)	2 (0.4)	9 (8.6)	5	24 (16.2)
Ni	9 (1.2)	8 (1.2)	7 (2.4)	9 (5.8)	4 (1)	3 (1.6)
Fe	95 (88)	34 (20)	42 (22)	38 (22)	16 (12)	17 (12)
Mn	246 (54)	251 (22)	260 (28)	21 (14)	10	24 (14)
No. sample[a]	4–8	4–8	4–8	3–5	2	2–9
			1973			
Zn	28.2 (3.8)	26.5 (5.6)	25.8 (6.2)	30.8 (32.8)	70.9 (41.2)	19 (20.6)
Cu	2 (1.4)	3 (2.8)	4 (5)	13 (11.8)	5 (2.6)	31 (16)
Ni	8 (0.4)	8 (0.4)	8 (0.6)	4 (2.6)	3 (1.4)	8 (3.4)
Fe	63 (18)	35 (8)	58 (42)	34 (36)	18 (6)	51 (22)
Mn	281 (50)	264 (20)	261 (28)	38 (16)	83 (11.6)	— —
No. samples[a]	5–7	6–7	5–8	4–9	3–13	5–8
			1972–73			
Pb	2	2	2	21	20	11
Cd	0.4	0.4	0.4	0.6	0.5	0.7
No. samples	7–10	9–10	8–10	10–13	11–14	13–14

[a]The number of samples analyzed for each heavy metal is reported in Beamish and VanLoon 1977.

outlet waters were highest in the early winter, probably as a result of high concentrations in snow. Precipitation was an important source of copper to the lake and watershed. The uptake of copper in soils, vegetation, or lake sediments possibly maintained a lower concentration in the lake than in the precipitation.

Concentrations of iron were extremely variable, possibly as a result of the difficulty in preserving and maintaining samples for analysis. Inlet concentrations were consistently higher than samples from other areas and iron deposits were observed on rocks in the inlet. Thus, precipitation and natural sources may contribute to the lake concentrations of iron. The concentration of iron in the lake was lower than the ELA lakes (Tables 3, 5) and thus does not appear to have been greatly altered by the fallout of acid and heavy metals.

Determinations of lead and cadmium were not made as routinely as the other heavy metals. The combined 1972 and 1973 concentrations of lead (Table 5) indicated that the inlet, lake, and outlet concentrations were low but slightly higher than the ELA lakes. Concentrations in the precipitation were much higher than lake concentrations or arctic snow (Table 3). Because the event precipitation samples were similar to the precipitation station determinations, apparently the elevated precipitation concentrations were real. Low concentrations in the lake indicated that lead was being removed either in the watershed or lake sediments or both.

Cadmium concentrations were also higher than in the ELA lakes (Tables 3, 5). Mean precipitation concentrations were slightly higher than lake concentrations and precipitation was apparently a contributor of cadmium to the lake. Some extremely high concentrations were measured in precipitation (1.8 and 2.7 $\mu g/\ell$) that were approaching lethal concentrations of 5 $\mu g/\ell$ for aquatic crustaceans (Biesinger and Christensen 1972).

OTHER SUBSTANCES

Mean concentrations of sulfates were higher in the lake, inlet, and outlet than in the ELA lakes and mean concentrations remained unchanged throughout this study (Tables 3, 6). Precipitation station concentrations averaged about one half of the lake concentrations and were similar to average event precipitation samples. Average surface snow concentrations of sulfate in 1972 and 1973 were almost identical but were lower than concentrations from the precipitation station. Precipitation could be an important supplier of sulfate to the lake; however, sulfate was also apparently supplied from sources other than precipitation.

TABLE 6. Mean (±2 SE) concentrations of other substances sampled.

	Inlet	Lake	Outlet	Precipitation		Surface snow
				Station	Event	
			1972			
SO_4	10.8 (1)	10.7 (.4)	10.9 (.6)	5.5 (1.2)	8.8 (5.4)	3.5 (.8)
Cl (*mg/ℓ*)	0.9 (.2)	1.1 (.4)	1.0 (.2)	0.7 (.2)	0.6 (.2)	0.7 (.2)
K (*mg/ℓ*)	.26 (.06)	.26 (.04)	.26 (.04)	.13 (.06)	.13 (.04)	.12 (.02)
Na (*mg/ℓ*)	.51 (.08)	.44 (.08)	.45 (.06)	.27 (.18)	.28 (.24)	.21 (.04)
Ca (*mg/ℓ*)	2.43 (.2)	2.35 (.2)	2.39 (.2)	.5 (.42)	.55 (.26)	.53 (.46)
Mg (*mg/ℓ*)	.66 (.12)	.64 (.12)	.62 (.08)	.09 (.06)	.18 (.12)	.10 (.02)
Cond ($\mu mhos/cm^3$)	31.6 (2.2)	32.5 (.4)	33.5 (1)	39 (16.4)	36.5 (23.4)	20.8 (5.4)
TDS (*mg/ℓ*)	19.9 (6.2)	18.3 (5)	21.1 (6.8)	23.8 (16.4)	7.3 (5.6)	6.2 (3.2)
TSS (*mg/ℓ*)	1.3 (1)	0.7 (.4)	1.1 (.6)	4.0 (4.6)	22.4 (25)	12.6 (11)
No. samples[a]	7–9	8–10	8–10	5–10	5–6	5–14
Total CO_2 (*μmol/ℓ*)	22.8 (10.6)	24.2 (9.2)	23.3 (9.4)	—	—	—
No. samples	5	5	5			
			1973			
SO_4 (*mg/ℓ*)	10.6 (.8)	10.7 (.6)	10.5 (.6)	4.5 (1)	5.6 (2.2)	34 (1.4)
Cl (*mg/ℓ*)	0.7 (.2)	0.7 (.2)	0.8 (.4)	0.7 (.2)	0.6 (.2)	1.7 (1.4)
K (*mg/ℓ*)	.26 (.04)	.27 (.04)	.25 (.02)	.11 (.02)	.13 (.08)	.09 (.02)
Na (*mg/ℓ*)	.45 (.02)	.47 (1)	.42 (.02)	.30 (.2)	.2 (.16)	.59 (.64)
Ca (*mg/ℓ*)	2.00 (.34)	1.97 (.28)	1.93 (.34)	.25 (.08)	.68 (.66)	.20 (.2)
Mg (*mg/ℓ*)	.56 (.08)	.65 (.12)	.57 (.06)	.04 (.02)	.08	.08 (.02)
Cond ($\mu mohs/cm^3$)	32.9 (2.2)	31.5 (1.8)	32.4 (2)	29.3 (14.8)	43 (6.4)	33.2 (6.4)
TDS (*mg/ℓ*)	21.8 (6.6)	18.3 (5.2)	25.0 (2.4)	19.7 (14.2)	5.5 (1)	10.2 (9.2)
TSS (*mg/ℓ*)	1.6 (.8)	1.2 (.8)	1.0 (.2)	13.4 (6.8)	5.3 (3)	4.6 (2)
No. samples[a]	6–8	6–8	6–10	3–9	2–10	4–5
Total CO_2 (*μmol/ℓ*)	39.3 (27)	44.9 (89.8)	48.5 (21.8)	—	—	—
No. samples	4	7	4			

[a]The number of samples analyzed for each substance is reported in Beamish and VanLoon 1977.

Chloride concentrations were similar to ELA lakes and were similar in the inlet, lake, outlet, and precipitation samples (Tables 3, 6). Mean chloride concentrations were slightly lower in 1973 than in 1972. Chloride concentrations in the precipitation were only slightly higher than the northern Canadian snow sample (Table 3).

Mean potassium concentrations were similar to the ELA mean (Tables 3, 6). There was little variation in the mean concentrations of all samples between 1972 and 1973. Precipitation concentrations of potassium and sodium were about one half of the lake concentrations and were higher than the concentrations from the northern snow samples (Table 3). Mean sodium concentrations in the inlet, lake, and outlet were approximately one half of the ELA mean (Tables 3, 6).

Mean concentrations of calcium were significantly lower ($P \leq 0.05$) in the lake and inlet in 1973 than in 1972 (Table 6). Outlet concentrations were also lower in 1973 but the differences were not significant. In 1972 the mean concentrations of calcium from all three types of precipitation samples were similar and the mean concentration from the precipitation station was significantly higher ($P \leq 0.05$) than the 1973 mean. The mean precipitation concentrations were about 5 times the concentrations measured in the northern snow sample (Table 3). However, precipitation did not appear to be an important supplier of calcium to the lake as lake concentrations were about 5 times higher than precipitation means. Mean lake concentrations were similar to the mean ELA concentration.

Magnesium concentrations were similar to ELA concentrations (Table 6). Precipitation concentrations were well below mean lake concentrations and did not appear to be major contributors to the lake concentration of magnesium. There was no change in the lake concentration from 1972 to 1973.

TABLE 7. Comparison of chemical composition of an event rain and of runoff during the event. (November 11, 1973).

Constituent	Rain	Run-off[a]	Run-off[b]	Run-off[c]	Run-off[d]
pH	4.2	3.8	3.8	4.0	4.4
SO_4 (*mg*/ℓ)	4.0	12	14.4	10.6	9.2
Cl (*mg*/ℓ)	.8	.4	1	.6	.6
Na (*mg*/ℓ)	.2	.2	.2	.3	.2
K (*mg*/ℓ)	.1	.2	.2	.1	.7
Mg (*mg*/ℓ)	ND	.1	.1	.2	.2
Ca (*mg*/ℓ)	.1	.5	.5	.4	1.0
Cond (*μmohs/cm*)	37	97	106	74	48
Fe (μg/ℓ)	18	69	128	57	37
Cu (μg/ℓ)	6	14	6	18	4
Zn (μg/ℓ)	120	110	135	100	185
Pb (μg/ℓ)	42	18	9	29	5
Ni (μg/ℓ)	3	121	19	21	18
Cd (μg/ℓ)	.8	.7	.8	1.1	1.0
Mn (μg/ℓ)	20	20	50	20	100

[a]Bedrock cliff face exposed to the S.E.
[b]Exposed bedrock cliff face.
[c]Bedrock cliff face exposed to the N.W.
[d]Soil covered.

Conductivity, total dissolved solids (TDS), and total suspended solids (TSS) mean concentrations were similar in the inlet, lake, and outlet in 1972 and 1973 (Table 6). TDS were slightly higher in the inlet and outlet probably as a result of increased turbulence in the shallow water. TDS concentrations from mean precipitation stations were higher than the event samples. Since there was no indication of contamination of the precipitation by other substances, the increase in TDS probably resulted from additions of organic material such as pollen.

Major ion concentrations from event precipitation samples and precipitation stations were similar indicating that contamination or excessive evaporation had not seriously altered the precipitation station samples.

Carbon dioxide concentrations were low as would be expected in such acid water (Table 6). Mean total CO_2 concentration in ELA lakes was about 150 μmol/ℓ at a pH of 6–7 (D. W. Schindler unpublished data). Mean CO_2 concentrations were significantly higher in 1973 in the lake and outlet than in 1972 ($P \leq 0.05$).

Nitrate (NO_3) was measured as 550 μg/ℓ in one event rain sample and 570 and 550 μg/ℓ in two snow samples.

RUNOFF ANALYSIS

Runoff from soilless, almost vertical rock faces (20–30 m high) was more acidic than rain (Table 7). Runoff from soil-covered bedrock was slightly less acidic than the rain. Sulfate ion concentrations were much higher in the runoff samples than from the event rain sample. Sulfate concentrations in the runoff samples were highest from the samples of lowest pH suggesting that sulfuric acid or sulfuric acid-producing substances had accumulated on the rock surfaces during the period of no rainfall. Other major ion concentrations (Na, K, Ca, Mg, and Cl) were unchanged or changed very slightly from the runoff of rock surfaces. The runoff from soil had higher concentrations of potassium and calcium than rain samples. The conductivity of runoff from rock surfaces was double to triple the event precipitation concentration, while there was little change in the conductivity of the soil runoff. Runoff concentrations of managnese, cadmium, and zinc from rock surfaces were similar to event rain concentrations; however, concentrations of manganese and zinc were increased in the runoff from soil. Nickel was 6–7 times more concentrated in all the runoff samples than in the precipitation event. Copper was unchanged in two runoff samples but double to triple the event concentration in two of the rock runoff samples. Iron varied between 2 and 5 times more concentrated in all the runoff samples.

Concentrations of lead in runoff were lower than in the precipitation event. The greatest reduction in lead concentration occurred in the soil runoff. This uptake of lead corroborates the previous observation that lead concentrations in Lumsden Lake were much lower than the pre-

cipitation concentrations, possibly because of accumulation of lead in the watershed or lake sediments.

Discussion

The decrease in lake pH in 1972 from 5.2 to 4.7 from January to December represents a real uptake of hydrogen ion and this increase in acid content was calculated to be closely associated with additions of acid from atmospheric fallout. Three other nearby lakes showed this increase in acidity in 1972 (Fig. 4), confirming that considerable fallout of acid occurred in this area in 1972. In 1973 there was no net change in pH in the lake over the 12-mo period. Precipitation loadings of acid were significantly ($P \leqslant 0.05$) lower than in 1972 and the predicted change in 1973 was from 4.8 to 4.7. As in 1972 the predicted change was very close to the observed change. All of the three lakes that were monitored as part of another study (Fig. 4) decreased in acid content during 1973, confirming that a reduction in acid loading did not occur only in the vicinity of Lumsden Lake.

Clearly the acid loading calculations are approximations of the actual concentrations being added. All acid that fell out onto the lake surface and surrounding drainage area and all acid that entered from the inlet, in excess of the lake concentration, was considered to be capable of directly reducing the pH of the lake. Changes in the acidity of precipitation falling on soils prior to entering the lake or changes within the lake were not considered. The relative importance of some of these problems was reduced by the choice of the study area. The surface area of the lake accounted for 21% of the drainage area (including the lake) and much of the area surrounding the lake consisted of exposed quartzite slopes. Some lakes in the area first showed signs of becoming acidified in the early 1960s (Beamish and Harvey 1972); thus, acid fallout probably had occurred in the area for some time allowing for a build up of acids in the soils. The lake was already acidic at the time of the study and probably many of the organisms normally found in this lake had been removed or reduced in abundance by earlier periods of low pH. Thus, many of the factors that might normally modify the acidity of precipitation may be of reduced importance in this study. Because the sampling program necessitated using the calendar year to calculate budgets, much of the December precipitation, which was mostly snow, contributed to the next year's budget. Errors caused by this method of calculation were minimized because acid loads during December did not fluctuate greatly. Finally, the calculations of the acid budget were based on bulk precipitation samples which presumably collected dry and wet fallout. Dry fallout was demonstrated to occur, and the use of bulk collectors to calculate acid fallout probably describes a "maximum" budget for the lake. While the importance of some of these factors in the acidification process can be disputed, the good correlation between the actual measured change in pH of the lake and the predicted change for 1972 and 1973 must be regarded as more than fortuitous and clearly demonstrates a close dependence and immediate response of the pH of this lake to atmospheric loading of acid.

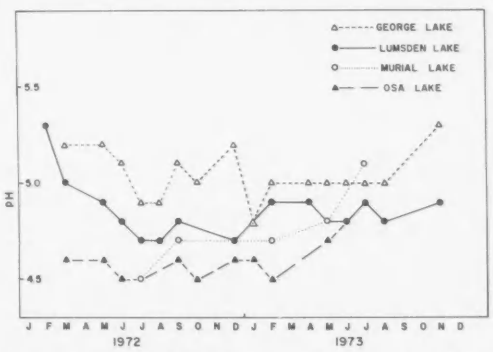

FIG. 4. pH of Lumsden and three nearby lakes during the study period.

Atmospheric fallout also contributed to the loading of heavy metals to the lake. Increased zinc concentrations were closely associated with pH and were similar to concentrations found in acid lakes in Europe and the United States (Beamish and VanLoon unpublished data; Hörnström et al. 1973; Schofield 1976). The similarity of response of lakes from different areas and the association of zinc concentration with pH suggest that increased mobilization of zinc in acid waters as well as atmospheric fallout may be important factors controlling zinc concentrations in acid lakes. The one runoff sample also indicated that increased mobilization may occur in soil runoff.

No significant linear relationship between pH and manganese was found for samples collected during the study from Lumsden Lake and three other lakes combined. Manganese concentrations were significantly higher ($P \leqslant 0.05$) in Lumsden and other nearby lakes, than in the precipitation. Manganese concentrations were also consistently high in the acidic lakes in Europe and the United

States. Despite the absence of a relationship between pH and manganese in this study, the high concentrations of manganese in acid lakes appears to result from increased mobilization from sediments or drainage area or both. The fivefold increase in manganese concentration between runoff samples from soils and event rain or rock runoff supports the conclusion that increased mobilization of manganese in waters of low pH is an important factor controlling manganese concentrations in acid lakes.

Concentrations of sodium, potassium, magnesium, or chloride were similar to concentrations found in the ELA lakes and these major ions did not appear to have been altered by the acidification process, to the same extent that sulfate, hydrogen, and perhaps calcium ions have been altered. As would be expected alkalinity was greatly reduced in Lumsden Lake in comparison with less acidic lakes.

Copper and nickel concentrations were higher in Lumsden Lake than in samples collected from acidified Swedish lakes (Beamish and VanLoon 1977). It is known that the smelters close to Lumsden Lake emit large quantities of nickel and copper as well as sulfur dioxide (Beamish 1974). The occurrence of elevated concentrations of these metals only in the waters in Lumsden and other nearby lakes (Beamish et al. 1975a; Beamish et al. 1975b) is strong evidence that much of the material that falls out into these lakes originates locally.

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Reexamination of the Carotenoid Pigments of the Unicellular Blue-green Alga *Agmenellum quadruplicatum*

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ANTIA, N. J., AND J. Y. CHENG. 1977. Reexamination of the carotenoid pigments of the unicellular blue-green alga *Agmenellum quadruplicatum*. J. Fish. Res. Board Can. 34: 659–668.

A previous claim for the occurrence of antheraxanthin and the absence of typical cyanophycean xanthophylls in *Agmenellum quadruplicatum* has been disproven. This phycocyanin-producing prokaryote showed chlorophyll *a*, β-carotene, zeaxanthin, one myxoxanthophyll-like, and one myxol-like carotenoid as the major pigments. Traces of keto and other carotenoids were observed, one of which appears to be phoenicopterone or a *cis*-echinenone. No antheraxanthin and chlorophyll *b* or *c* were detected under all culture conditions tested. Algal chlorosis from culture aging was caused by the consecutive loss of phycocyanin and chlorophyll, but the carotenoid composition was much less affected and there was no gain of secondary or keto carotenoids. We concluded that this alga is a typical member of the Cyanophyceae in terms of pigment chemotaxonomy.

ANTIA, N. J., AND J. Y. CHENG. 1977. Reexamination of the carotenoid pigments of the unicellular blue-green alga *Agmenellum quadruplicatum*. J. Fish. Res. Board Can. 34: 659–668.

Nous réfutons une affirmation antérieure concernant la présence d'anthéraxanthine et l'absence de xanthylles typiques des cyanophycés chez *Agmenellum quadruplicatum*. Ce prokaryote produisant de la phycocyanine montre comme pigments majeurs la chlorophylle *a*, le β-carotène, la zéaxanthine, un caroténoïde ressemblant à de la myxoxanthophylle et un ressemblant à du myxol. Nous observons des traces de céto et autres caroténoïdes, dont l'un a été provisoirement identifié comme phœnicoptérone ou un *cis*-échinénone. Nous n'avons pas détecté d'anthéraxanthine ni de chlorophylle *b* ou *c* dans toutes les conditions de culture essayées. La chlorose algale accompagnant le vieillissement des cultures est causée par la perte consécutive de la phycocyanine et de la chlorophylle, mais la composition en caroténoïdes est beaucoup moins affectée et il n'y a pas de gain de caroténoïdes secondaires ou céto. Nous en concluons que cette algue est un membre typique des Cyanophycaea en termes de chimiotaxonomie des pigments.

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PARSONS (1961) reported the anomalous occurrence of the epoxyxanthophyll antheraxanthin (Fig. 1F) as a major carotenoid and the absence of typical cyanophycean xanthophylls in *Agmenellum quadruplicatum*. A recently compiled summary (Hertzberg et al. 1971) of the carotenoid composition of 28 species of the Cyanophyceae showed no epoxy carotenoids in this algal class other than the rare occurrence of the furanoxy-carotene mutatochrome (Fig. 1E) in trace amounts, suggesting that cyanophycean epoxidation of xanthophylls is virtually absent. According to this summary, the Cyanophyceae characteristically produce one or more members of the following structurally defined families of xanthophylls: (A) β-carotene-based ring-keto carotenoids such as echinenone and canthaxanthin (Fig. 1A); (B) β-carotene-based ring-hydroxylated carotenoids such as β-cryptoxanthin, zeaxanthin, caloxanthin and nostoxanthin (Fig. 1B); (C) γ-carotene-based oxygenated carotenoids such as myxoxanthophyll and other myxol-derived glycosides (Fig. 1C); (D) lycopene-based oxy-

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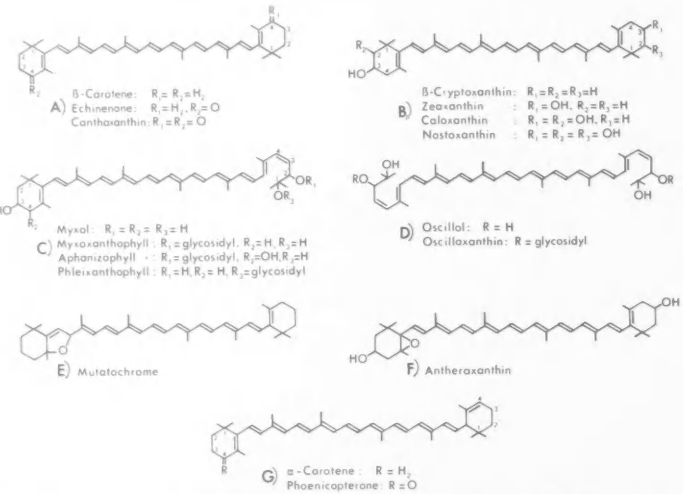

FIG. 1. Structural representation of cyanophycean xanthophyll families A, B, C, D, and other types of carotenoids (E, F, G) discussed in the text.

genated carotenoids such as oscillaxanthin and other oscillol-derived glycosides (Fig. 1D). A previous claim of an additional family of allenic type xanthophylls in four species (Stransky and Hager 1970) was subsequently shown to be structurally erroneous, and members of this group (caloxanthin, nostoxanthin) were found to belong to the above-designated family B (Buchecker et al. 1976). Most cyanophycean species produced one or more representatives of at least two of these xanthophyll families, and some showed those of all the families (Hertzberg et al. 1971). However, the occurrence of various combinations of the different xanthophyll families in an algal species seems to be quite unpredictable, and the similarities discerned between certain related species (Healey 1968) may be fortuitous.

Since *A. quadruplicatum* is a unicellular cyanophyte belonging to the order Chroococcales (Stanier et al. 1971), it is pertinent to consider the xanthophyll families found in other members of this taxonomic order. A *Gloeobacter* species, lacking thylakoids, showed the family combination A–D with its reported xanthophyll composition of echinenone and oscillaxanthin-type glycosides (Rippka et al. 1974), while *Synechococcus elongatus* indicated the combination A–B–C with its production of echinenone, zeaxanthin, and myxoxanthophyll (Stransky and Hager 1970). Interestingly, the entire set of xanthophyll families A–B–C–D appears in *Microcystis aeruginosa* containing echinenone, canthaxanthin, cryptoxanthin, zeaxanthin, myxoxanthophyll, aphanizophyll, and oscillaxanthin (Stransky and Hager 1970). Furthermore, different strains of *Anacystis nidulans* may produce different sets of xanthophyll families, since two strains (Stransky and Hager 1970; Buchecker et al. 1976) apparently showed only members of the B family (cryptoxanthin, zeaxanthin, caloxanthin, nostoxanthin) sometimes accompanied by unidentified xanthophylls (Smallidge and Quackenbush 1973), while a third strain (Halfen and Francis 1972) produced the family combination A–C xanthophylls (echinenone, canthaxanthin, myxoxanthophyll and its 4-keto derivative) with no trace of family B. In any case, irrespective of strain or species differences, antheraxanthin has never been recorded from any chroococcalean member nor from any other order of the Cyanophyceae, apart from Parsons' (1961) report on *A. quadruplicatum*.

Parsons' (1961) identification of β-carotene and chlorophyll *a* as the major photosynthetic pigments (ignoring the phycobilins) of *A. quadruplicatum* is in accord with previous findings on all Cyanophyceae. In view of the recent discovery of chlorophyll *b* and no phycobilin pigment in a *Synechocystis* species (Lewin and Withers 1975), we considered the probability that Parsons' antheraxanthin anomaly may be another case of chemotaxonomic deviation in atypical members of the Cyanophyceae, which required confirmation. Accordingly, we have reinvestigated the

entire pigment composition of *A. quadruplicatum* under several culture conditions, and the results are reported in this communication.

Materials and Methods

Algal culture

Strain PR-6 of *Agmenellum quadruplicatum* (Menegh.) Brébisson was obtained in axenic condition, during 1964, from the marine blue-green algal culture collection of Van Baalen (1962) and has since been maintained in a liquid seawater-based medium under continuous illumination (Antia and Kalmakoff 1965) for several years but more recently using sequential transfer cycles of 11 wk storage in darkness at 20°C followed by 3 wk of growth in light (Antia and Cheng 1970). This strain is believed to be the same as that used by Parsons (1961). However, the identification of this strain by Drouet and Daily (1956) has been questioned by Stanier et al. (1971) and there is also considerable doubt about its being a true marine form (R. Y. Stanier personal communication).

The alga was axenically cultured at 20°C in 5 ℓ of seawater, buffered with TRIS at pH 7.9–8.1, and enriched with 1.75 mM nitrate, 0.15 mM phosphate, 0.5 mM silicate, excess of vitamins (B_{12}, thiamine, biotin) and EDTA-chelated trace-metal ions (Fe, Mn, Zn, Mo, Cu, Co). The culture was aerated (5% CO_2-enrichment) with magnetic stirring and continuously illuminated (cool-white fluorescent lamps, ca. 10–15 klx) for 15 days from inoculation, when it achieved an opaque, deep-green appearance with a cell count of 44.9×10^6 cells/ml. Pigment-analysis aliquots were aseptically withdrawn at 5 and 15 days from initiation of the culture. The culture was then incubated for 7 days in total darkness with continuous stirring and occasional aeration, at the end of which period it had retained its opaque green appearance and virtually comparable cell count (42×10^6 cells/ml), when a third sample was removed for pigment examination. The rest of the culture was then replaced in light with stirring and occasional aeration. Aging of the culture was followed by visual examination of its color, which changed progresssively from deep bluish-green to yellow–green 28 days later (with cell count at 188×10^6 cells/ml) when a fourth pigment sample was removed. The incubation of the culture was now continued in light without aeration until the coloration was a bleached cream-yellow (with cell count at 152×10^6 cells/ml) about 69 days from initiation, when it was discontinued and the remaining culture was completely used up for a fifth pigment analysis. The viability of this bleached culture was established by a subculture test into fresh growth-medium which showed rapid recovery of healthy pigmented growth. The different pigment aliquots taken were labeled according to culture age (rounded off in weeks) as (i) 1 wk, (ii) 2 wk, (iii) 3 wk, (iv) 7 wk, (v) 10 wk, with the understanding that the 3-wk and older samples included 1 wk of incubation in total darkness.

Pigment Analysis

Each culture-age sample was immediately centrifuged, the clear colorless supernate discarded, and the pelleted algal material exhaustively extracted with methanol. The unextracted residue was dried, weighed, and its color noted visually for presence or absence of the blue-hued phycocyanin. A small portion of the total methanol extract was diluted to a known volume and its absorption spectrum (310–710 nm) was quantitatively recorded to estimate the total chlorophyll and carotenoids by standard methods (Strickland and Parsons 1972). The methods were modified to correct for methanol as the solvent, with specific extinction coefficients for chlorophyll *a* ($72\ \ell \cdot g^{-1} \cdot cm^{-1}$ at 666 nm) and total carotenoid ($220\ \ell \cdot g^{-1} \cdot cm^{-1}$ at 476 nm, expressed as combined carotene–zeaxanthin equivalents) being derived from the reports of Tett et al. (1975) and Chapman (1965), respectively. Where required, another portion of the methanol extract was saponified overnight (under N_2 at room temperature) with one tenth its volume of 60% aqueous KOH, and the chlorophyll-free pigments were taken up in ether. The rest of the original extract was evaporated to dryness in vacuo, redissolved in minimal amount of methanol–benzene (1:1), and stored (cold under N_2) in ready state for chromatographic analysis. The saponified ether extract was similarly treated prior to chromatography.

All chromatographic analyses were effected on thin-layer plates, using the previously outlined procedure of stepwise fractionations of partially separated pigment groups by repeated chromatography on the appropriate adsorbent–solvent combinations offering progressively greater resolution for each pigment group (Cheng et al. 1974). The same procedure was used for preparative chromatography, with bands of pigment mixtures placed on thin-layer plates of adsorbent thickness enhanced up to 1 mm. The identification of pigments was based on their absorption spectra, chromatographic mobilities, and direct comparison with authentic specimens when available. The gross proportions of the resolved carotenoids were estimated by comparing optical densities of equivalent eluted solutions at their respective principal λ_{max}, assuming equal specific-extinction coefficients. Some of the purified carotenoids were characterized by chemical tests for keto or epoxy groups and for *cis–trans* isomerization as described before (Cheng et al. 1974). Authentic zeaxanthin, lutein, and echinenone were kindly supplied by Dr B. C. L. Weedon and canthaxanthin by Hoffman–LaRoche, Ltd. Authentic diatoxanthin and alloxanthin were isolated from *Isochrysis galbana* and *Chroomonas salina*, respectively.

Results

A typical pigment separation from a culture sample is illustrated in Fig. 2, where the initial chromatogram produced four purely carotenoid-containing clusters (I, II, V, VI) and two chloro-

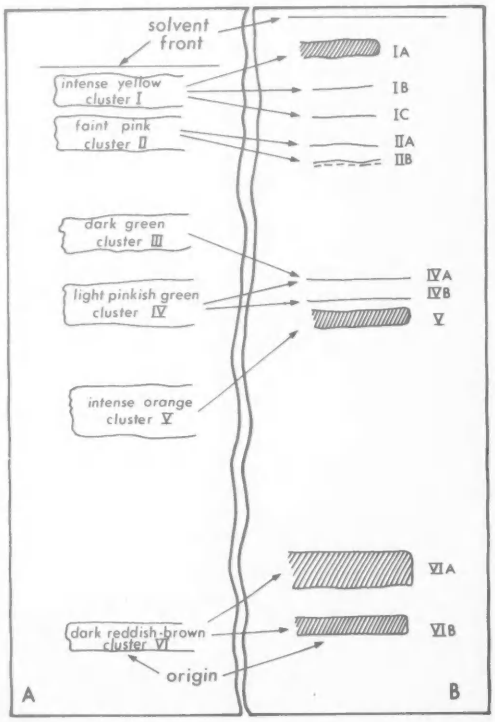

FIG. 2. Typical ascending thin-layer chromatograms (on silica-gel G plates) of *Agmenellum* methanol-extracted pigments (A) before saponification, (B) after saponification. Developing solvent systems used: plate A, petroleum ether: ethyl acetate: diethyl amine (58: 30: 12 vol/vol); plate B, *n*-propanol: hexane (15:85 vol/vol). Pigments identified: IA, β-carotene; IB, unknown carotene; IC, lycopene; IIA, mutatochrome; IIB, phoenicopterone ± echinenone; IVA, (*iso*) cryptoxanthin; IVB, hydroxyechinenone; V, zeaxanthin; VIA, myxol-group xanthophyll 6A; VIB, myxol-group xanthophyll 6B. The green pigment in clusters III and IV (plate A) was identified as chlorophyll *a* and *a'*, respectively. Carotenoid IVA appeared either in cluster III or IV, depending upon their separation.

phyll-dominated clusters (III, IV) containing minor carotenoids. After further fractionation, the components of each cluster were tentatively identified as follows; their spectra and results from chemical tests are listed in Table 1. Cluster I revealed β-carotene as a major carotenoid accompanied by traces of lycopene and an unknown carotene. Cluster II showed traces of mutatochrome, phoenicopterone, and echinenone, and their occurrence was erratic with respect to culture age. Clusters III and IV showed traces of cryptoxanthin (or *iso*cryptoxanthin) and hydroxyechinenone accompanying large amounts of chlorophyll *a* or its isomer (presumed to be an artefact). The carotenoid components of these clusters were best resolved after removal of the chlorophyll by saponification. Cluster V showed virtually pure zeaxanthin as a major carotenoid. The most polar cluster VI revealed two major xanthophylls, one of which appeared to be similar to myxoxanthophyll and the other a less polar myxol-derived xanthophyll. No carotenoid with the spectral, chromatographic, and chemical (positive epoxide test) properties of antheraxanthin was detected.

The identity of zeaxanthin was confirmed by co-chromatography with an authentic specimen in several adsorbent–solvent systems. It showed spectroscopic and chromatographic difference from authentic lutein. The acidification treatment showed a slight shift (Table 1) in its absorption maxima, indicating that it could not be an epoxide such as antheraxanthin. The *cis–trans* isomerization test also confirmed its identity with zeaxanthin and difference from authentic diatoxanthin and alloxanthin.

The structure of the keto carotenoid (tentatively) identified as phoenicopterone (Fig. 1G) was deduced from its spectrum (λ_{max} 452, petroleum ether) and that of its $NaBH_4$-reduction product (λ_{max} 472, 445, 425). Although chromatographically indistinguishable from echinenone, our keto carotenoid showed consistent spectrometric difference from authentic echinenone (λ_{max} 457) and its reduction product (λ_{max} 476, 450, 428). The spectra of phoenicopterone and its reduction product (Fig. 3) are in reasonable accord with those expected from 4-keto-α-carotene as reported for this keto carotenoid from *Astasia ocellata* (Thomas et al. 1967) and *Haematococcus pluvialis* (Czygan 1968a). However, we must point out that there is much uncertainty in our identification of phoenicopterone, since the keto carotenoid and its reduction product showed relatively elevated absorption in the 330–390 nm region (Fig. 3) suggesting that they could be *cis*-isomers of echinenone and its reduction product, respectively. Synthetic 15, 15′-*cis*-echinenone was reported to show absorption maxima at 454 and 349 nm (Akhtar and Weedon 1959), but other *cis*-isomers have not been previously characterized. Our experimental evidence appears insufficient to distinguish between phoenicopterone and *cis*-echinenone.

The two most polar xanthophylls, comprising cluster VI in Fig. 2, showed absorption spectra (Table 1; Fig. 4) indicative of myxol-derived carotenoids (Hertzberg and Liaaen-Jensen 1969, 1971; Francis et al. 1970). Both these xantho-

TABLE 1. Spectral characteristics of lipoid pigments found in *Agmenellum* and compared with some literature values; * indicates single broad peak covering the stated wavelength range or stretching some nanometers around the approximate λ_{max} values reported in the literature; ~ inflexion or shoulder (instead of a peak) at the wavelength following this symbol; Bz, benzene; EtOH, ethanol; MeOH, methanol; Me_2CO, acetone; PE, petroleum ether; Pyr, pyridine.

Pigments	% carotenoid	Absorption maxima (λ_{max}) wavelengths (nm) in the solvents shown
β-Carotene	26	~426, 452, 478 (EtOH)
Unknown carotene	trace	442, 466, 487 (EtOH)
Lycopene	trace	446, 470, 504 (EtOH)
Mutatochrome	1	405, 427, 450 (EtOH)
Phoenicopterone (?)	2	450–453* (PE) $\xrightarrow{NaBH_4}$ ~425, 445, 472 (PE); 452–456* (EtOH) $\xrightarrow{}$ ~426, 448, 474 (EtOH)
Echinenone (?)	trace	454–460* (PE); 460–466* (EtOH)
Chlorophyll *a*	—	384, 416, 431, 532, 584, 618, 665 (EtOH)
β-(or *iso*) Cryptoxanthin	1	~426, 450, 476 (EtOH)
Hydroxyechinenone (?)	trace	454–462* (EtOH)
Zeaxanthin	40	~427, 452, 479 (EtOH) $\xrightarrow{acid}$ ~424, 447, 475 (EtOH); ~430, 453, 480 (Me_2CO); ~438, 464, 492 (Bz)
Myxol group		
Xanthophyll 6A	12	448, 474, 505 (EtOH); 448, 475, 506 (Me_2CO); 464, 492, 526 (Pyr)
Xanthophyll 6B	18	448, 474, 504 (EtOH); 450, 476, 505 (Me_2CO); 464, 493, 524 (Pyr)
		Literature[a–g] *values*
Echinenone[a]		ca. 461* (EtOH)
3′-Hydroxyechinenone[a]		ca. 462* (EtOH)
4′-Hydroxyechinenone[a]		ca. 460* (EtOH)
Cryptoxanthin[a]		~428, 449, 473 (EtOH)
*Iso*cryptoxanthin[b]		~425, 450, 477 (EtOH)
Antheraxanthin[c]		446, 474 (EtOH) $\xrightarrow{acid}$ 426, 454 (EtOH)
Myxoxanthophyll[a,d]		448, 473, 503 (EtOH); 465, 493, 528 (Pyr)
Aphanizophyll[a,e]		445, 472, 502 (EtOH); 450, 476, 507 (Me_2CO)
Phleixanthophyll[f,g]		~449, 473, 503 (MeOH); 465, 493, 526 (Pyr)

[a]Stransky and Hager 1970; [b]Healey 1968; [c]Hager and Meyer-Bertenrath 1967; [d]Hertzberg and Liaaen-Jensen 1969; [e]Hertzberg and Liaaen-Jensen 1966; [f]Schlegel 1958; [g]Hertzberg and Liaaen-Jensen 1967a.

phylls were scarcely affected by saponification, $NaBH_4$, or brief acid treatment, suggesting that they lacked ester, keto, and epoxy groups. Their chromatographic mobilities were widely divergent on polyamide layers in reversed-phase systems (Fig. 5), where the faster moving xanthophyll 6A compared favorably with trihydroxylated or tetrahydroxylated carotenoids such as neoxanthin and vaucheriaxanthin. This comparison suggested that 6A may not be a glycoside but another derivative, possibly a hydroxylmyxol or myxol itself. On the other hand, the slower moving xanthophyll 6B showed virtually no mobility in all the chromatographic systems tested, in addition to being extremely difficult to dissolve or elute in organic solvents excepting the most polar ones such as pyridine. These properties suggest 6B to be a glycoside such as myxoxanthophyll or aphanizophyll (Hertzberg and Liaaen-Jensen 1969, 1971), but the possibility is not precluded that it could also turn out to be another type of myxol-based glycoside such as phleixanthophyll (for structure, see Fig. 1C) reported from a mycobacterium (Schlegel 1958; Hertzberg and Liaaen-Jensen 1967a) although hitherto not recorded for blue-green algae. In the absence of authentic specimens and the appropriate facilities for rigorous chemical examination, we are reluctant to hastily infer the precise identity of the xanthophylls 6A and 6B, but our results leave little doubt that the former is a myxol-derived aglycone and the latter a myxol-based glycoside.

Among the major pigments found in *Agmenellum*, β-carotene and chlorophyll *a* were positively identified by direct comparison with authentic specimens, while phycocyanin was visually recognized from the blue color of the methanol-insoluble algal residue left after total extraction

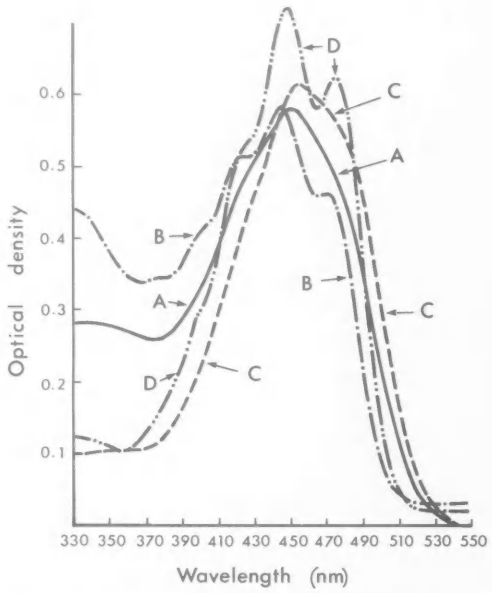

FIG. 3. Absorption spectra (in petroleum ether) of *Agmenellum*-produced phoenicopterone (A), its $NaBH_4$-reduction product (B), authentic echinenone (C), and its corresponding reduction product (D).

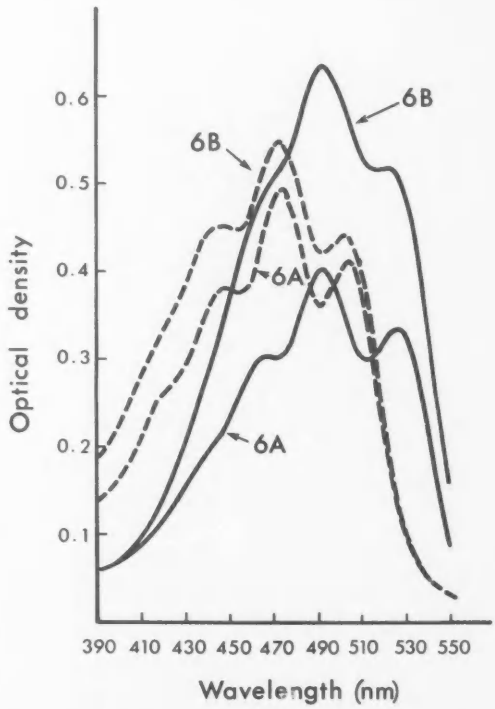

FIG. 4. Absorption spectra of myxol-group xanthophylls 6A and 6B in ethanol (shown by broken line) and pyridine (shown by solid line).

of chlorophyll and carotenoids. Separate experiments involving treatment of the whole algal material with aqueous buffers at pH 7–8 have shown this blue color to accompany a water-soluble protein precipitated by trichloroacetic acid; since this property is characteristic of the known phycobiliproteins (Ó hEocha 1965), we have inferred this pigment from its color to be predominantly composed of a phycocyanin without further examination.

The minor carotenoids were obtained in too little quantity for adequate characterization. The identities of lycopene, mutatochrome, echinenone, hydroxyechinenone, and crypto-(or *iso*crypto) xanthin were tentatively inferred from their chromatographic mobilities (before and after saponification) and comparison of their absorption spectra with those reported in the literature (see Table 1). In the absence of authentic specimens, we were unable to distinguish the assignment of cryptoxanthin or *iso*cryptoxanthin to a chromatographically defined monohydroxylated carotenoid with β-carotene type spectrum.

The effects of culture aging on the total pigment composition are summarized in Table 2. Phycocyanin appeared virtually absent by the time the culture was 7 wk old and chlorophyll *a* declined to a trace at 10 wk. The older culture samples showed additional chlorophyll-colored spots on chromatograms, but careful spectroscopic examination established these to be isomerization or dephytylated products of chlorophyll *a* (Michel-Wolwertz and Sironval 1965; Bacon 1966; Seliskar 1966) and there was no evidence of chlorophyll *b* or *c* production at any stage of culture aging. The total carotenoid content also declined progressively with aging, but its composition remained remarkably constant, with β-carotene, zeaxanthin, and the myxol-type xanthophylls persisting as major carotenoids throughout the aging process. There was no significant gain of keto carotenoid content from the aging, in contrast to the age-related development of these carotenoids known for certain members of the Chlorophyceae (Czygan 1968b; Kleinig and Czygan 1969; McLean 1967, 1968). Furthermore, we observed no formation of antheraxanthin at any culture age and not even after incubating the culture for 1 wk in darkness. The reversible epoxidation of zeaxanthin to antheraxanthin and violaxanthin from alternate exposure to light and

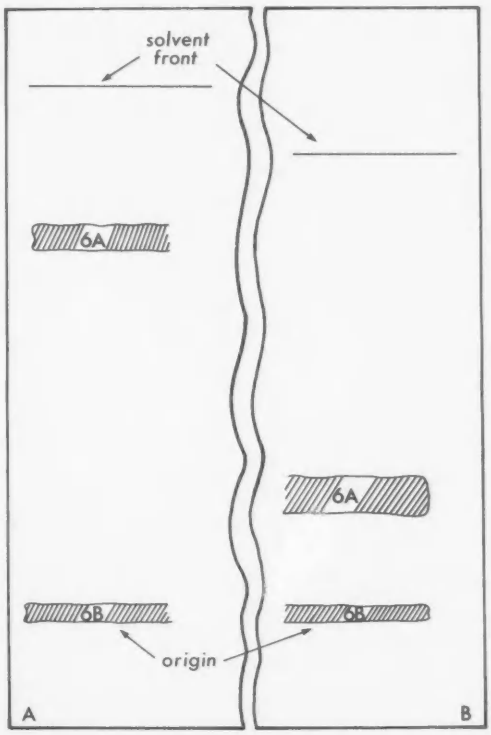

FIG. 5. Reversed-phase ascending chromatograms of myxol-group xanthophylls 6A and 6B on thin layers of Polyamide 6 developed with mixtures of methyl ethyl ketone, methanol, water in the proportions (A) 5: 5: 1 (vol/vol), (B) 3: 3: 2 (vol/vol).

darkness has been recorded for certain green algae (Hager and Stransky 1970) but was not detected in the blue-green and red algae previously tested (Stransky and Hager 1970). Our observations confirm that *Agmenellum* is incapable of xanthophyll epoxidation and antheraxanthin formation, irrespective of culture age or a light→darkness→light cycle.

Discussion

In view of several cases of carotenoid misidentification reported in the literature (see reviews of Goodwin 1971, 1974), we have been cautious in inferring the identity of the pigments isolated in the present investigation. We are certain of our identification of the major pigments (i.e. phycocyanin, chlorophyll *a*, β-carotene, zeaxanthin, and two myxol-based xanthophylls) but that of the minor pigments (apart from distinguishing the keto carotenoids) will need confirmation from rigorous chemical study. Furthermore, the complete structures of the two myxol-based xanthophylls (one resembling myxoxanthophyll and the other its aglycone) could not be established within the scope of this investigation. We note from his λ_{max} values (450, 479 nm) that Parsons (1961) may have actually isolated zeaxanthin (authentic λ_{max}: 449, 478 nm) instead of his supposed antheraxanthin, whose absorption maxima should be at significantly lower wavelength (444, 472–473 nm) according to Davies (1965) and Hager and Meyer-Bertenrath (1967). We also suspect that Parsons (1961) may have inadvertently "lost" the myxol-based xanthophylls by choosing a pigment-extraction solvent (i.e. hexane) in which these xanthophylls are virtually insoluble. These considerations may account for the odd xanthophyll composition of *Agmenellum* obtained by this author.

Our revised pigment picture leaves little doubt that *Agmenellum* is a typical cyanophyte producing the combination of xanthophyll families A–B–C (Fig. 1), albeit with a weak tendency for keto carotenoid biosynthesis even on aging. In these respects, the alga resembles more closely the pigment composition of *Synechococcus* rather than those of *Microcystis* or *Anacystis*, if we consider only the chroococcalean members of the Cyanophyceae (Stransky and Hager 1970). Our detection of traces of lycopene has a parallel in the study of Francis and Halfen (1972) on *Oscillatoria princeps*, while traces of mutatochrome, hydroxyechinenone, and isocryptoxanthin have been randomly observed in several species of blue-green algae (Hertzberg and Liaaen-Jensen 1967b; Healey 1968; Stransky and Hager 1970; Francis and Halfen 1972). The only unusual pigment feature of *Agmenellum* is the likelihood of a residual capacity for α-cyclization (i.e. formation of the α-ionone end-group) during carotenoid biosynthesis. This possibility is suggested by our doubtful detection of phoenicopterone, which is presumably formed by C_4-oxidation of α-carotene (see Fig. 1G), and we urge future investigators to verify our findings by large-scale *Agmenellum* cultivation and rigorous chemical examination of the minor carotenoids.

The effects of culture aging on pigment composition are attributed to the progressive depletion of inorganic nitrogen in the culture medium following the exponential growth of the alga. The bleached (chlorotic) appearance of blue-green algae from nitrogen starvation has been shown to result from the sequential loss of phycocyanin and chlorophyll (Allen and Smith 1969; De Vasconcelos and Fay 1974; Katoh and Ohki 1975), in which respects the aging behaviour of

TABLE 2. Summary of culture-aging effects on the pigment composition of *Agmenellum quadruplicatum*.

Pigment		Approximate[a] culture age (from continuous illumination interrupted by 1 wk of total darkness[b])				
		1 wk (L)	2 wk (L)	3 wk (L→D)	7 wk (L → D → L)	10 wk
Cell count ($\times 10^9$ cells/ℓ)		31.7	44.9	42.0	188	152
Chlorophyll *a* (μg/10^9 cells)		62.2	40.9	35.8	14.1	0.092
Phycocyanin[c]		+++	++	+	−	−
Total carotenoid (μg/10^9 cells)		24.5	24.5	22.4	7.1	0.259
β-Carotene	% of total carotenoid	26.2	30.7	26.0	23.1	27.6
Keto and minor carotenoids		0.4	2.3	4.7	3.7	trace
Zeaxanthin		52.4	38.1	39.7	46.3	53.4
Myxol-group xanthophylls 6A and 6B		21.0	28.9 (44:56)[d]	29.6 (40:60)[d]	26.8 (38:62)[b]	19.0

[a]Note that the culture samples were actually taken at 5, 15, 22, 50, and 69 days from inoculation.

[b]The periods of illumination (L), 1 wk of darkness (D) followed by restoration of illumination (L) are shown in sequence under each age.

[c]Plus and minus scores are used to denote the presence or absence, respectively, of this pigment, as deduced from visual observations (see text).

[d]These ratios show the proportions of xanthophylls 6A: 6B, where they could be accurately determined.

Agmenellum was comparable. However, while the aging also caused a considerable decline in the total carotenoid content, the proportions of major carotenoids remained remarkably constant within a range of minor fluctuations, and there was no increase in secondary (usually keto) carotenoids known to accompany aging (or nitrogen deprivation) of certain green algae (Hager and Stransky 1970; Donkin 1976). Aging effects on the carotenoid composition of other blue-green algae have not been previously investigated or reported. Among the effects of other variables previously studied, we noted that raising the culture temperature of *Anacystis nidulans* from 20 to 38°C caused a sevenfold increase in the echinenone content, accompanied by a twofold increase of β-carotene and myxoxanthophyll; thus, there was a substantial gain of a keto carotenoid from the growth at the higher temperature (Halfen and Francis 1972). Since our strain of *Agmenellum* is known to be capable of good growth at temperatures as high as 39°C (Van Baalen and Marler 1963), we suggest that its cultivation at such temperatures might offer a good means of enhancing keto carotenoid production from the barely detectable levels obtained by us at 20°C; such enhanced production is required to isolate enough of these carotenoids for confirming their chemical identities, especially that of our supposed phoenicopterone.

We hope that our suggestions for further research on *Agmenellum* carotenoids will prove profitable to future investigators, since we are unable to continue this study beyond the stage reported here.

Acknowledgments

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Recent Sedimentary History of a Eutrophic Reservoir: Long Lake, Washington

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THOMAS, S. R., AND R. A. SOLTERO. 1977. Recent sedimentary history of a eutrophic reservoir: Long Lake, Washington. J. Fish. Res. Board Can. 34: 669–676.

Through abatement of present high nutrient loadings to the reservoir and preservation of sediment integrity, we predict that eutrophic Long Lake will experience a rapid recovery after the City of Spokane, Washington, establishes an advanced wastewater treatment plant with phosphorus removal. Two sediment cores were zoned into annual layers according to profile concentrations of organic matter, nitrogen, phosphorus, and diatom frustules. Sedimentation rate was 26 $mm \cdot yr^{-1}$ between 1958 and 1973. Below the 25 mm-depth, phosphorus and diatom profiles were most obvious because clay particles, entering the reservoir during spring runoff, covered the biological material precipitated the previous year. This mechanism effectively prevents internal recycling of nutrients, particularly phosphorus, from the bottom sediments.

Key words: reservoir, sediment cores, phosphorus, nitrogen, organic matter, diatom frustules, clays, internal nutrient recycling, recovery

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Nous prédisons que, grâce à la diminution des présents apports élevés d'éléments nutritifs dans le réservoir et la préservation de l'intégrité des sédiments, le lac Long eutrophe se rétablira rapidement une fois que la ville de Spokane, Washington, aura installé une usine de traitement avancé des eaux usées avec enlèvement du phosphore. On a déterminé dans deux carottes de sédiments des couches annuelles selon les concentrations de profils de matière organique, d'azote, de phosphore et de frustules de diamtomées. La vitesse de sédimentation fut de 26 $mm \cdot an^{-1}$ entre 1958 et 1973. Au-dessous de la profondeur de 27 mm, les profils de phosphore et de diatomées sont les plus évidents du fait que les particules d'argile, pénétrant dans le réservoir au moment du ruissellement printannier, recouvrent le matériel biologique précipité l'année précédente. Ce mécanisme empêche effectivement un recyclage interne des éléments nutritifs, en particulier le phosphore, des sédiments du fond.

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HIGH nutirent input from the City of Spokane's primary sewage treatment plant is the major cause of eutrophication in Long Lake, an impoundment of the Spokane River. Also, nutrient release from sediments during anoxic periods is thought to be partly responsible for the reservoir's trophic state (Soltero et al. 1973, 1974, 1975). It is unknown whether the sediments will retain or release accumulated nutrients after the city has initiated secondary sewage treatment with phosphorus removal.

This paper describes the quantity of organic matter, nitrogen, phosphorus, manganese, iron, and zinc present in sediment cores taken from Long Lake. Diatom analysis from two cores and other selected data are used to determine the annual sedimentation rate and changes in biological production since 1958. In addition, the depositional pattern of phosphorus and clays are used to predict the potential for retention or release of phosphorus from the sediments.

Materials and Methods

STUDY AREA

Long Lake is approximately 24 km northwest of Spokane, Washington (Fig. 1). Long Lake Dam, completed in 1915, is in Lincoln County at 47°50′12″N, 117°50′20″W. Morphometric data for the reservoir at maximum capacity (elevation 468.2 m) are given below:

Max length	35.4 km
Max effective length	5.8 km
Max width	1.1 km
Max effective width	1.1 km
Mean width	571.8 m
Max depth	54.9 m
Area	208.4×10^5 m²
Vol	304.9×10^6 m³
Shoreline length	74.3 km
Bottom grade	0.15

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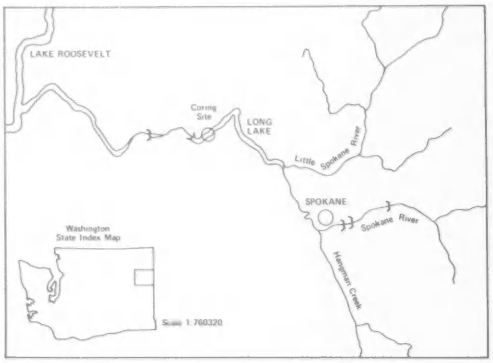

FIG. 1. Map of Long Lake detailing the study area.

Since 1958 the reservoir has received effluent from the City of Spokane's primary sewage treatment plant via the Spokane River. Prior to 1958 the river received untreated sewage.

A sampling station, approximately 8 km upstream from the dam, was chosen for coring because the upper reaches of the reservoir bottom are periodically scoured by high runoff whereas the lower portion lies in a narrow-graded channel.

CORE SAMPLES

Ten sediment cores were taken on July 31, 1974 in 30 m of water. Upon visual examination of consolidation and laminar features, four representative cores were selected for analysis. The cores were taken using a Phleger gravity corer. A plastic liner, 60 cm long, internal diameter 3.5 cm, was inserted into the corer to contain the sample. Each core was prepared for extrusion by freezing the plastic liner and its contents for 30 min, then heating the liner until the sediment column could be extracted. All cores remained consolidated upon extrusion, and vertical contamination due to compaction or expansion was not obvious during sample preparation. Sampling was at 5-mm intervals with a device similar to the one described by Fast and Wetzel (1974). Each sample was oven-dried for 24 h at 105°C, then milled to a fine consistency (sieve opening of 2 mm).

ANALYSIS OF CORES

Subsamples of known weight for each section were analyzed for organic matter (loss on ignition at 550°C for 30 min) and total nitrogen (Kjeldahl method) as described by the APHA et al. (1971). Total phosphorus concentration was determined by the persulfate digestion procedure (EPA 1974). All data were expressed on a dry sediment weight basis.

Cores 2 and 4 were selected to estimate absolute diatom numbers in each section. Slides were prepared using the evaporation tray technique described by Battarbee (1973) and Moore (1973). Morphological units, as described by Stockner and Benson (1967), representing each diatom taxon were enumerated until a minimum of 100 fields of view or 200 frustules per slide were recorded. The average number of frustules per dry sediment weight was calculated utilizing the necessary area ratios, magnification, and field-of-view factors.

Sediment clay mineralogy of core 4 was determined using a Picker Diffractometer, Model No. 3488L. One sample was prepared by compositing subsamples taken from sections throughout the length of the core where maximum nutrient concentration occurred. An additional sample was similarly prepared from sections of minimum nutrient concentration.

Manganese, iron, and zinc were brought into solution by nitric–perchloric acid digestion and analyzed by direct aspiration into an atomic absorption spectrophotometer. All data were expressed on a dry sediment weight basis.

Results

ORGANIC MATTER, NITROGEN, PHOSPHORUS, AND DIATOMS

The sediment–water interface of the cores was represented by a light-brown floc approximately 5 mm thick. The floc graded into an underlying green-colored ooze approximately 20 mm thick. Below 20 mm the sediments were more consolidated and contained hydrogen sulfide, which suggests that the oxidized microzone may have extended to a depth of 25 mm. Sediment below 25 mm was characterized by two distinct types of layering. Beds of pale laminae (light-to-dark brown) 20–40 mm thick were separated by dark laminae (dark brown to blue-black) 5–10 mm in alternating sequence. Below 20 cm, layering was less distinguishable.

Each core was zoned based on the continuity between alternating high and low points between organic matter, nitrogen, phosphorus, and diatom horizons (Fig. 2). The vertical distance between horizons was similar throughout the length of all cores. However, the ratio of organic matter to nitrogen and to phosphorus varied, and higher concentrations of organic matter, nitrogen, and phosphorus were determined below the 25 mm depth in all cores. Horizon resolution below the 25 mm depth was most obvious for the phosphorus and diatom profiles.

High numbers of sedimented frustules appeared to correspond with periods of high diatom production (June–October) in the overlying waters and low numbers of sedimented frustules with periods of low diatom production (November–May) (Fig. 3). The mean diatom cell volume at station 1 during July 1972 was 50% less than that observed in July 1973 and this was reflected in the sediments by the number of diatom frus-

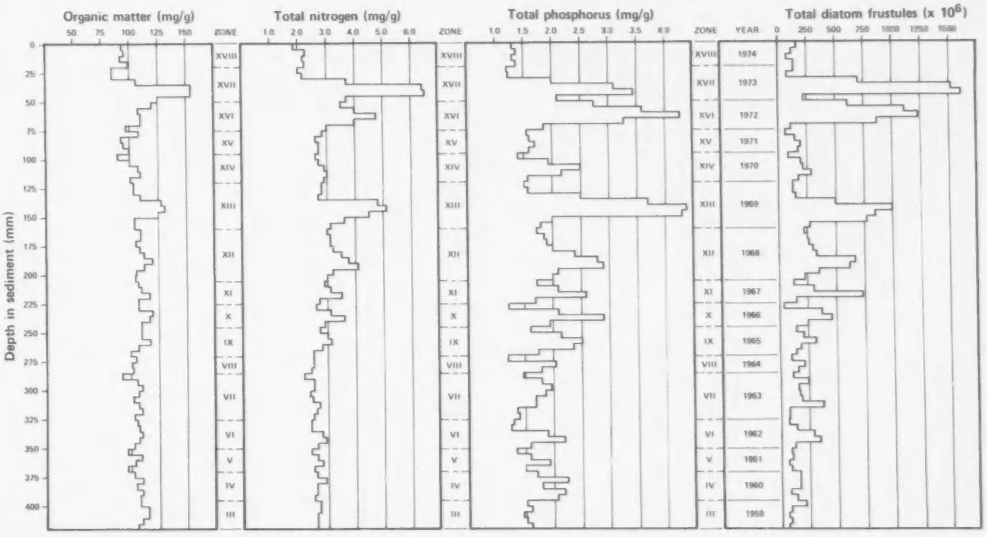

FIG. 2. Stratigraphy of organic matter, total nitrogen, and total phosphorus concentrations, and sedimented diatom frustules of core 2 expressed on gram dry weight basis. The stratigraphy of core 4 is similar to that of core 2.

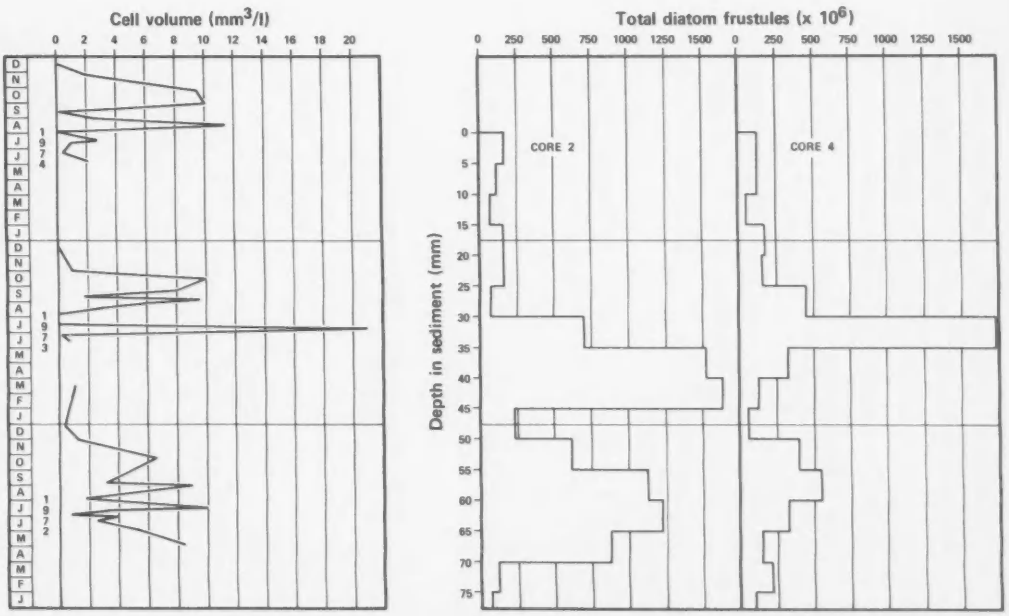

FIG. 3. Mean diatom cell volumes in the euphotic zone by date (from Soltero et al. 1973, 1974, 1975), and sedimented diatom frustules, expressed on gram dry weight basis, in the upper 75 mm of cores 2 and 4.

tules at the 60- and 35-mm depths, respectively. Periods of low diatom production appeared to be represented at sediment depths of 15, 50, and 75 mm. Dating of the cores was therefore based on the assumption that subsamples with high diatom counts represented sediment accumulation during the biologically productive time of year and that low counts represented accumulation during biologically inactive periods. Dating of each subsample in this manner showed that approximately 2.5 mm of sediment was incorporated for each month between January 1972 and July 1974.

An annual sedimentation rate of approximately 26 $mm \cdot yr^{-1}$ for all cores was determined by assigning years to corresponding zones and dividing the sum of the overall mean sediment depth between zones II and XVII by 16 yr. Zones I and XVIII were not used in the calculation of the overall mean.

Zone	Sediment accumulation (mm)	Year
XVIII	21.2	1974
XVII	28.8	1973
XVI	35.0	1972
XV	23.8	1971
XIV	26.2	1970
XIII	30.0	1969
XII	28.8	1968
XI	27.5	1967
X	30.0	1966
IX	23.8	1965
VIII	22.5	1964
VII	30.0	1963
VI	20.0	1962
V	25.0	1961
IV	26.2	1960
III	23.3	1959
II	15.0	1958
I	15.0	1957
Mean	26.0	

Twenty-six genera and 34 species were identified from a total of 50,200 diatom frustules. The assemblages found in the sediments appeared to be a mixture of diatoms from different habitats influent to (Williams 1975) and within the reservoir (Gasperino 1974). *Fragilaria crotenensis* and *Melosira italica* were the most abundant taxa and together comprised over 69% of the total frustule count (table below). The six taxa listed below accounted for 83.1% of the frustules identified from the sediment. (Values are based upon counts from all subsamples of cores 2 and 4.)

Rank	Taxon	Abundance (%)
1	*Fragilaria crotonensis*	41.0
2	*Melosira italica*	28.7
3	*Melosira granulata*	6.8
4	*Synedra ulna*	3.6
5	*Asterionella formosa*	1.8
6	*Tabellaria fenestrata*	1.2

With the exception of *F. crotonensis*, in 1958 taxa counts were comparatively low (Fig. 4). *Fragilaria* numbers declined in 1971 as did *Melosira* numbers; however, in 1972 the rate of increase in *M. italica* numbers was greatest. By 1973, *Fragilaria* and *Melosira* numbers had generally increased above previous levels, whereas *Synedra ulna*, *Asterionella formosa* and *Tabellaria fenestrata* numbers declined.

Generally, increased sediment concentrations of organic matter, nitrogen, and phosphorus between 1958 and 1973 were accompanied by increased diatom counts (Fig. 5). The combined numbers of *Melosira* reflected the profile shown for the Order Centrales and *Fragilaria*, *Synedra*, *Asterionella*, and *Tabellaria* reflected the profile for the Order Pennales. Significant correlations based on zone means existed between diatom numbers and nitrogen ($r = 0.95$; $P = 0.005$), diatom numbers and phosphorus ($r = 0.75$; $P = 0.005$), and diatom numbers and organic matter ($r = 0.53$; $P = 0.05$).

Clay Minerology, Manganese, Iron, and Zinc

The predominant mineral in the cores other than silicon dioxide (alpha quartz) was illite clay. In the composite sample of the sections that possessed low nutrient concentrations, the illite peaks (002, 004 and 110) were 60% higher than in the composite sample of sections with high nutrient concentrations. This indicates an increased abundance of illite at points of low nutrient concentration (Dr W. K. Steele, Department of Geology, Eastern Washington State College, Cheney, Wash. 99004, personal communication).

Alternating high and low points for the manganese profile appeared to correspond to assigned zones, whereas iron and zinc profiles did not (Fig. 6). The mean manganese concentration within zones increased in the upper sediments whereas iron concentrations remained relatively stable throughout the length of the core. Zinc concentrations decreased in the upper sediments.

Using zone means the concentrations of phosphorus, manganese, and iron for core 4 were statistically compared. A correlation existed be-

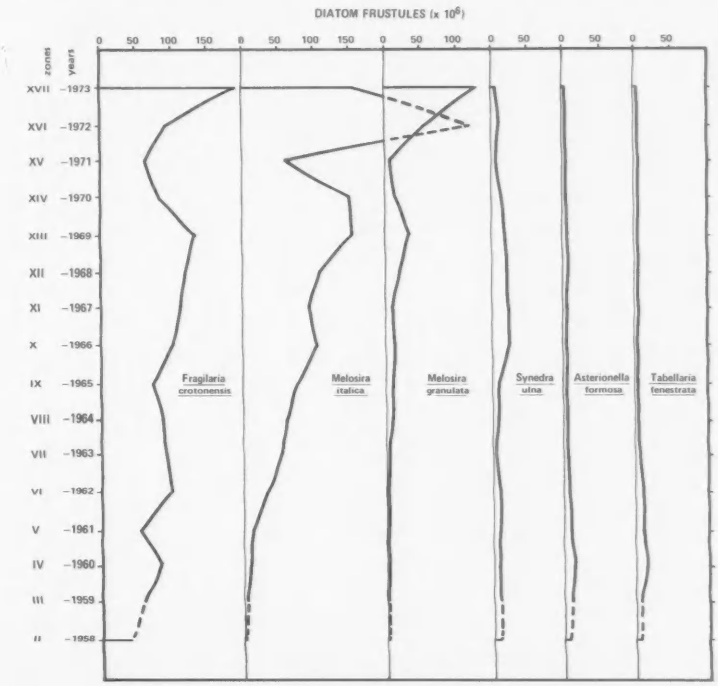

FIG. 4. Zone and yearly means of the six most abundant sedimented diatoms for cores 2 and 4 expressed on gram dry weight basis.

tween phosphorus and manganese ($r = 0.44$; $P = 0.05$); however, the relationships between phosphorus and iron ($r = 0.08$), and iron and manganese ($r = 0.10$) were not significant.

Discussion

Diatom enumeration has shown that production has increased approximately sevenfold since 1958. Increased *Fragilaria* and *Melosira* numbers and a concomitant decline in *Synedra*, *Tabellaria* and *Asterionella* indicated that increased nutrient loadings have been more favorable for the former and less favorable for the latter diatoms. *Fragilaria crotonensis* often causes nuisance blooms in enriched reservoirs, and its association with eutrophication is well documented (Lund 1954, 1969; Stockner and Benson 1967; Holland 1968; Holland and Beeton 1972; Javornicky and Kamarkova 1973). Although *M. italica* and *M. granulata* ranked second and third in abundance, respectively, they exhibited the greatest rate of increase since 1958. *Melosira italica* favors moderately but not strongly oligotrophic or eutrophic temperate or subarctic lakes (Lund 1954). *Melosira granulata* is usually characteristic of warm, turbulent, often strongly eutrophic lakes (Lund 1954; Schelske and Stoermer 1972), but is rare or missing in oligotrophic waters (Lund 1962). *Melosira italica* and *M. granulata* can survive for long periods on anoxic sediments in a physiological resting stage (Lund 1954, 1955). Changes in *Melosira* populations in one season, or a series of seasons, may influence the size of the maxima in the next because of the importance of the resting stage in providing a reserve of live cells during the summer that serve as "inoculum" for renewed growth (Stockner and Lund 1970; Lund 1971).

Chemical analyses of the sediment suggested that increased nutrient loading and the resulting diatom production was the result of the larger volume of sewage inffluent to the primary sewage treatment facility. In 1958 when the sewage treatment facility went "on line" there had been 44,280 side sewer permits issued and 734.22 km of sewer line constructed. By 1973 both figures had increased approximately 1.4 times, which resulted in a similar increase in mean daily flow to the sewage treatment plant. Since 1958 the concentration of nitrogen and phosphorus in the

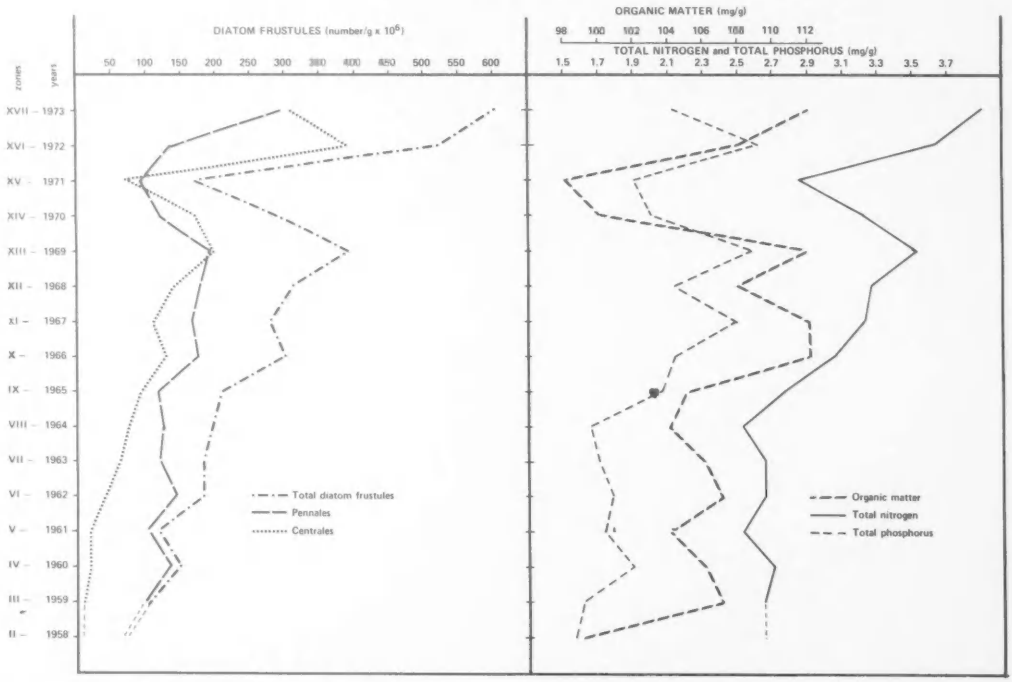

FIG. 5. Zone and yearly means of sedimented diatom frustules, organic matter, nitrogen, and phosphorus for cores 2 and 4 expressed on gram dry weight basis.

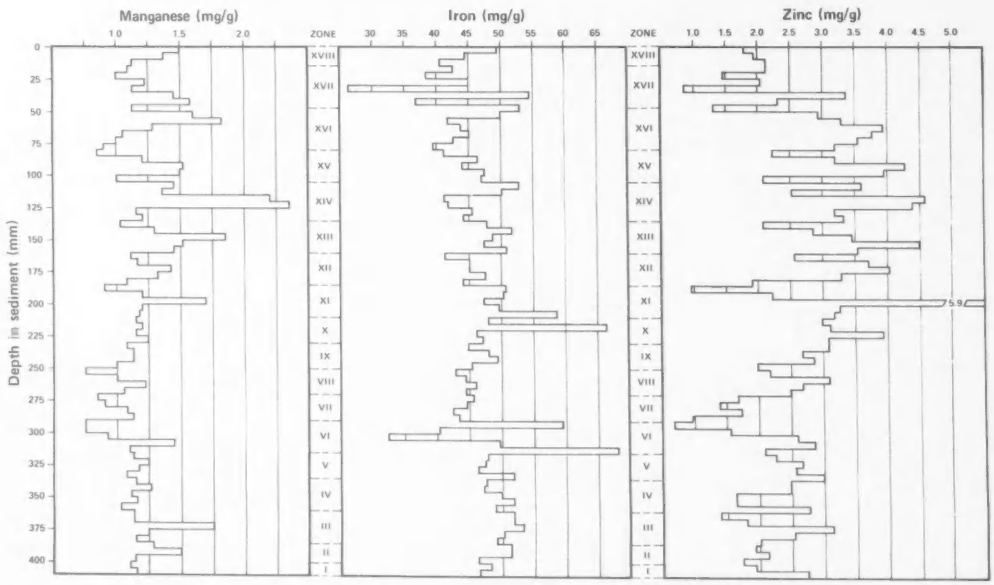

FIG. 6. Stratigraphy of manganese, iron, and zinc concentrations of core 4 expressed on gram dry weight basis.

sediments have increased about 1.4 times and reflects the increased sewage flow.

The sedimentary organic matter content showed the least increase of the assessed parameters; it appeared to be predominately autochthonous (primarily diatoms) and represented a relatively stable product once it had undergone the initial stages of diagenesis (Krumbein and Garrels 1952; Kleerekoper 1953; Gray and Kemp 1970; Burns and Ross 1972).

Manganese, iron, and phosphorus concentration profiles were not closely related and from the depositional pattern it is assumed that manganese and iron compounds do not play an important role in the precipitation of phosphorus to the sediments. Mackereth (1966) concluded that phosphorus deposition in oligotrophic Ennerdale Lake, England, was not influenced by variations in the iron and manganese cycle, but precipitation of phosphorus was largely biological and constant. Also, the manganese, iron, and phosphorus concentration profiles in oligotrophic Weber Lake, Wisconsin, were not related (Bortleson and Lee 1974) and it was concluded that iron was probably associated with clay minerals, which would provide "little opportunity" for phosphorus precipitation with iron compounds (Bortleson 1971).

Even though anoxic conditions caused by volatile solid stabilization in the hypolimnion and at the mud–water interface have prevailed in Long Lake during each year of study, nutrient budgets have shown that the reservoir was a phosphorus "sink" (Soltero et al. 1973, 1974, 1975). The mean nitrogen to phosphorus ratio of 1.5 also reflected the affinity of the sediments to trap and retain phosphorus. Sorption of phosphorus by lake muds, whether oxygen is present or not, can remove considerable amounts of phosphorus from lake water (Fitzgerald 1970; Serruya 1971; Schindler and Fee 1974).

Clay particles appear to be an important structural component of the sediments of Long Lake and are thought to play an important role in the distribution of phosphorus. During spring runoff (March–May) erosional activity in the basin brings into the reservoir heavy loads of suspended material (Soltero et al. 1973, 1974, 1975). As runoff subsides, suspended clay particles settle from the water column and eventually become incorporated over a layer containing high concentrations of phosphorus of biological origin that was laid down over a clay layer deposited the previous year. Thus, we hypothesize that the sedimented clays brought in during spring runoff have effectively sealed off the sediment–water interface each year and minimized mixing and vertical migration of phosphorus from the underlying sediment layers. The resolution of sedimentary phosphorus and diatom horizons in all cores support this argument. Based on this data, we predict that internal recycling of nutrients, patricularly phosphorus, from the bottom sediments will not actively contribute to the nutrient supply of Long Lake once secondary sewage treatment with phosphorus removal is initiated by the City of Spokane.

Acknowledgments

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Effects of Diet, Ambient Temperature, and Holding Conditions on Plasma Thyroxine Levels in Rainbow Trout (*Salmo gairdneri*)

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LEATHERLAND, J. F., C. Y. CHO, AND S. J. SLINGER. 1977. Effects of diet, ambient temperature, and holding conditions on plasma thyroxine levels in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 34: 677–682.

Rainbow trout (*Salmo gairdneri*) acclimated to 19°C and fed a pelleted diet of either 6% soybean oil or 6% tallow fat had similar plasma thyroxine levels (0.62 ± 0.10 and 0.69 ± 0.08 μg/100 ml, respectively), although fish acclimated to 7°C and fed the soybean diet gave significantly ($P < 0.05$) higher plasma thyroxine concentration (1.32 ± 0.13 μg/100 ml) than the group fed the tallow fat diet (0.95 ± 0.12 μg/100 ml). The plasma thyroxine concentration in the fish fed the soybean oil diet was significantly ($P < 0.01$) higher at 7°C than at 19°C. There was no significant difference between the two groups of fish given the tallow fat diet. Epithelial cell heights were largest in groups with the highest plasma thyroxine levels. Epithelial cell height in the two groups at 19°C were similar, but in both groups of fish at 7°C, the cell height was significantly greater than in either of the groups at 19°C ($P < 0.01$ for comparisons of the 19°C tallow fat diet with both groups of 7°C acclimated fish and comparison of the two groups on the soybean oil diet; $P < 0.05$ for comparisons of the 19°C, soybean oil diet with the group held at 7°C and given the tallow fat diet). Fish deprived of food for 40 days had a significantly ($P < 0.05$) lower plasma thyroxine concentration when compared with fish that had been fed daily. There was no significant diurnal variation in plasma thyroxine over the light phase of a 12 h light:12 h dark photoperiod, nor did the limitation of light entering the aquaria significantly affect plasma thyroxine values.

Key words: endocrine, thyroid, *Salmo gairdneri*, diet, environmental temperature

LEATHERLAND, J. F., C. Y. CHO, AND S. J. SLINGER. 1977. Effects of diet, ambient temperature and holding conditions on plasma thyroxine levels in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 34: 677–682.

Des truites arc-en-ciel (*Salmo gairdneri*) acclimatés à 19°C et nourris à un régime en boulettes contenant 6% soit d'huile de soya soit de suif ont des nivaux identiques de thyroxine plasmatique (0.62 ± 0.10 et 0.69 ± 0.08 μg/100 ml respectivement); cependant les poissons acclimatés à 7°C nourris au régime de soya donnent une concentration de thyroxine plasmatique significativement ($P < 0.05$) plus forte (1.32 ± 0.13 μg/100 ml) que le groupe nourri au suif (0.95 ± 0.12 μg/100 ml). La concentration de thyroxine plasmatique chez les poissons nourris à l'huile de soya est significativement ($P < 0.01$) plus élevée à 7°C qu'à 19°C. Il n'y a pas de différence significative entre les deux groupes de poissons nourris au régime de suif. Les cellules épithéliales sont les plus hautes chez les groupes possédant les plus hauts niveaux de thyroxine plasmatique. La hauteur des cellules épithéliales dans les deux groupes à 19°C est identique, mais dans les deux groupes de poissons à 7°C, la hauteur des cellules est significativement plus grande que dans l'un ou l'autre des groupes à 19°C ($P < 0.01$ dans le cas de comparaisons du régime de suif à 19°C avec les deux groupes de poissons acclimatés à 7°C et dans le cas de la comparaison des deux groupes nourris au régime d'huile de soya; $P < 0.05$ dans le cas des comparaisons du régime à l'huile de soya à 19°C avec le groupe maintenu à 7°C et nourri au régime de suif). Les poissons privés de nourriture pendant 40 jours ont une concentration de thyroxine plasmatique significativement ($P < 0.05$) plus basse que celle des poissons nourris quotidiennement. Il n'y a pas de variation nycthémérale significative dans la thyroxine plasmatique avec une photopériode de 12 h de clarté: 12 h de noirceur; la limitation de la lumière pénétrant dans les aquariums n'affecte pas non plus de façon significative les valeurs de la thyroxine plasmatique.

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THE study described here examines the effects of food deprivation, different diets, ambient temperatures, and holding methods on plasma thyroxine levels in rainbow trout (*Salmo gairdneri*).

Recent reviews have outlined problems associated with investigations of thyroid function(s) in teleosts (Gorbman 1969; Sage 1973). Many of the most commonly used methods of investigation, namely histology, labeled iodide incorporation, and thyroxine treatment have made meaningful interpretation of the findings difficult. For example, histologically "active" thyroids may well be symptomatic of a hyperthyroid or even goiterous condition if iodide is not available (Sonstegard and Leatherland 1976). Similarly, the rapid incorporation of radioactive iodide in these fish would also erroneously suggest a hyperactive thyroid condition. The converse method of investigation, namely the administration of thyroxine, may equally provide false information. For example, Eales (1974) showed that within a few hours trout that had been immersed in thyroxine solutions had chronic levels of plasma thyroxine; thus, the responses of fish to these extreme hyperthyroid levels may have little, if any, physiological meaning.

One approach that has not been widely applied in lower vertebrates (as noted by Packard et al. 1976) is to monitor changes in serum (or plasma) thyroxine in fish subjected to controlled experimental conditions. In this way changes in thyroxine levels may indirectly provide insight into processes in which thyroid hormones are involved. Recently, Narayansingh and Eales (1975a, b) have shown that thyroxine administration tends to promote mobilization of lipids in brook trout and to stimulate protein synthesis in brook and rainbow trout. Other authors (see review by Swift 1960, also Drury and Eales 1968) have demonstrated an apparent inverse relationship between ambient temperature and thyroid activity in some salmonid fishes. Thus, if the thyroid is indeed implicated in some way as a metabolite regulator and "temperature compensator" in fishes, changes in the levels of plasma thyroxine may be expected in fish fed different diets and maintained at different ambient temperatures. Results of experiments designed to test this hypothesis are described.

Materials and Methods

SUPPLY AND MAINTENANCE OF FISH

Fish for experiments 1, 2, and 3 were obtained from the Ontario Ministry of Natural Resources, Normandale Hatchery, and for experiment 4, from a local hatchery. The fish were maintained in flowing (2.5 exchanges of water/h) and aerated fresh water at the temperatures indicated in Table 1. Photoperiod was 12 h light:12 h dark.

DIETS

The control diet was 35% herring meal, 35% wheat middlings, 20% soybean meal, 6% soybean oil, 1% linseed oil, and 2% of a vitamin and 1% of a mineral premix modified slightly from that used by Cho et al. (1974). This diet was used for all experiments. An additional diet with 6% soybean oil replaced by 6% of prime tallow fat was used in experiment 3.

WEIGHT GAINS AND CARCASS COMPOSITION

Weight gains of the fish in experiment 2 were derived from weights at the start and finish of the experiment. Carcass lipid and protein compositions were measured by the Bligh and Dyer (1959) and Kjeldahl (reported in A.O.A.C. 1970) methods, respectively. Samples of five randomly selected fish from each experimental group were pooled, weighed, freeze-dried, and ground in a micromill. Duplicate portions of the dried fish were then used for moisture, lipid and protein determinations.

PLASMA THYROXINE ASSAY

Blood was collected in either ammonium heparinized capillary tubes or sodium heparinized "vacutainer" tubes after transection of the caudal peduncle, then centrifuged and the plasma stored at −40°C

TABLE 1. Effect of diet, water temperature and holding methods on plasma thyroxine levels in rainbow trout (*Salmo gairdneri*).

	Water Temp (°C)	No. fish	Plasma T4 (μg/100 ml)
Experiment 1			
killed at 0830	18	10	0.96±0.06[a]
killed at 1330	18	12	0.93±0.09
killed at 1830	18	13	0.86±0.03
Experiment 2			
covered tanks (100 lx)	15	10	0.93±0.09
uncovered tanks (600 lx)	15	19	0.70±0.06
Experiment 3			
soy oil diet	19	8	0.62±0.10
tallow diet	19	8	0.69±0.08
soy oil diet	7	7	1.32±0.13
tallow diet	7	8	0.95±0.12
Experiment 4			
starved	11	6	1.19±0.11
fed	11	5	2.48±0.53

[a]Mean ± SE.

until required. Thyroxine levels were assayed using the Ames Tetralute protein binding assay using the modification recommended to maximize the sensitivity of the assay (sheet No. 1-75-D). The assay used was essentially similar to that used by Higgs and Eales (1973) and Chan and Eales (1976). In our laboratory the assay sensitivity ranged from 0.1 to 0.2 μg/100 ml for rainbow trout plasma and coho salmon serum. Serum thyroxine levels (measured using this assay) increased significantly in rainbow trout 1–3 h after an intracoelomic injection of ovine TSH (Leatherland unpublished data). Moreover, plasma thyroxine levels were found to be significantly lower in rainbow trout immersed for 10 days in a 0.05% thiourea solution (Leatherland unpublished data) and serum thyroxine levels showed a progressive decline in coho salmon serum concomitant with goitre formation (Sonstegard and Leatherland 1976). Thus, the assay appears to adequately detect changes in the activity of the pituitary–thyroid axis in salmonid fishes. Interassay variability represented < 5% (expressed as percent retention of I^{125} on column) in four standard curves of assays performed at weekly intervals. Interassay variability was < 5% for 10 samples of trout plasma assayed on 2 consecutive days (Leatherland unpublished data). In the measurements made for this study, plasma samples to be compared were measured in the same assay batch using the same reagents.

Statistics

In experiment 2 the data of plasma thyroxine were compared by one-way analysis of variance and those of epithelial cell heights by one-way analysis of variance with subsamples. Individual means were compared with least significance difference. In experiment 2 and 4 the means were compared by Student's *t*-test.

Experimental Procedure

Experiment 1: A group of 100 fish was maintained for 60 days in an opaque container (69 ℓ) at 18°C and fed daily at 1, 3, 6, and 9 h after the onset of the photoperiod with ad libitum quantities of the control diet. Groups of 15–20 fish were killed at 1, 6, and 11 h after the start of the photoperiod. Where necessary plasma samples from one to three fish were pooled. Mean body weights (±SE) of the fish sampled were 58.1 ± 3.2, 55.2 ± 4.1, and 56.3 ± 3.1 g for fish killed 1, 6, and 11 h, respectively, after the start of the photoperiod.

Experiment 2: Two groups of 100 fish each were maintained for 180 days in identical opaque containers (69 ℓ) at 15°C. They were fed ad libitum as in experiment 1 with the control diet. One group was maintained in containers with transparent lids; light intensity at the water surface was 100 and 1600 lx, respectively. Mean body weights (±SE) for samples from covered and uncovered tanks were 45.8 ± 4.6 and 55.6 ± 6.5 g, respectively.

Experiment 3: A 2 × 2 factorial experiment was conducted in which groups of 100 fish were fed either a control diet or a control diet with 6% of the soybean oil replaced with tallow fat at 19°C (mean 18.87 ± 0.05°C) or 7°C (mean 7.20 ± 0.14°C). All groups were maintained in aquaria identical to those in experiment 1. They were fed ad libitum 4 times a day at 1, 3, 6, and 9 h after the onset of the photoperiod. Samples of fish from each group were killed at 112 days. Mean body weights (±SE) for the four groups of fish were 77.3 ± 6.6, 75.3 ± 3.4, 31.6 ± 2.1, and 29.9 ± 1.9 g for the 19°C soy, 19°C tallow, 7°C soy, and 7°C tallow diets, respectively.

Experiment 4: Two groups of 30 fish were maintained in identical 682-ℓ uncovered aquaria; one group was fed daily at the middle of the photoperiod with 1% body weight of the control diet, the second group received no food. Six fish from each group were killed at 40 days. Mean body weights (±SE) for fed and starved fish were 162.9 ± 18.3 and 155.5 ± 16.3 g, respectively.

Results

Experiment 1: There was no significant difference in the plasma thyroxine levels in the three groups of fish (Table 1).

Experiment 2: There were no significant differences in the plasma thyroxine concentrations in the two groups (Table 1).

Experiment 3: There was no significant difference between the plasma thyroxine levels in the two groups maintained at 19°C (Table 1). However, in the groups acclimated to 7°C the fish fed the soybean oil diet had significantly ($P < 0.05$) higher plasma thyroxine values compared with similar fish fed the tallow diet. Thyroxine levels were significantly higher in 7°C acclimated fish fed the soybean oil diet compared with both groups of fish at 19°C ($P < 0.01$ for soybean diet and $P < 0.05$ for tallow diet). There were no significant differences between the tallow fed fish maintained at 7°C and 19°C, although the plasma thyroxine levels were generally higher in the 7°C acclimated fish.

There was no significant difference between epithelial cells heights in the fish acclimated to 19°C (Table 2), but in both these groups the epithelial cell height was significantly less than that in the fish adapted to 7°C ($P < 0.01$ for comparisons of the 19°C tallow fat diet with both groups acclimated to 7°C and the two groups given the soybean oil diet; $P < 0.05$ for comparison of 19°C soybean oil diet with 7°C tallow fat diet).

Experiment 4: There was a significantly ($P < 0.05$) higher level of plasma thyroxine in the control fish compared with trout deprived of food for 40 days (Table 1).

TABLE 2. Effect of diet and temperature on thyroid epithelial cell height of rainbow trout.

Diet	Water temperature (°C)	No. fish	Thyroid epithelial cell (μm)
Soybean oil	19	8[a]	2.14±0.56[b,c]
Tallow fat	19	8	1.73±0.56[d]
Soybean oil	7	7	3.04±0.56
Tallow fat	7	8	2.89±0.56

[a]20 cell measurements made in each fish.

[b]Mean ± SE; SE calculated as

$$\sqrt{\frac{\text{experimental error mean square}}{\text{experimental error degrees of freedom}}}$$

(Steel and Torrie 1960).

[c]Significantly different from 7°C soy oil diet ($P < 0.01$) and 7°C tallow fat diet ($P < 0.05$).

[d]Significantly different from both 7°C groups ($P < 0.01$).

Discussion

Rainbow trout deprived of food for 40 days had plasma thyroxine levels significantly ($P < 0.05$) lower than those in fish fed 1% of their body weight each day. The lower thyroxine levels in starved (1–2 wk) trout (Higgs and Eales, 1973) compared with fed fish (Refetoff et al. 1970) led Packard et al. (1976) to suggest that food deprivation may have the effect of lowering plasma thyroxine levels. However, Eales (1974) reported lower plasma thyroxine levels in fed brook trout compared with starved fish and Chan and Eales (1976) reported thyroxine values "close to zero" in both fed and starved brook trout. Thus, the effect of food deprivation on plasma thyroxine is not clear. The discrepancies between data from different sources may represent species differences or be related to differences in lengths of starvation periods used. However, although there are significant differences between thyroxine levels in starved and control fish in these experiments, the hormone levels in both groups are closer to those reported by Higgs and Eales (1973) and Bromage et al. (1976) than they are to the rather high values obtained by Refetoff et al. (1970).

Since all the samples in this study were stored in a frozen state, the plasma thyroxine values given in Table 1 may be lower than the "true" values because Packard et al. (1976) found a 32% reduction in thyroxine readings in samples of hagfish plasma that had been frozen. However, the samples from comparable groups were always stored under the same conditions and thus differences within each experiment probably have physiological significance. Moreover, in preliminary studies there was no evidence of a significant loss of thyroxine in trout serum samples as a result of freezing (Leatherland unpublished data).

The findings from experiment 3 suggest that the type of diet and the ambient water temperature can effect significant changes in circulating thyroxine levels. The differences between the soybean oil and the tallow fat diets consisted of only a 6% change in the basic diet, well within the differences that could be encountered in hatchery conditions. Both diets were adequate to meet the requirements of 1% ω3 fatty acids as recommended by Castell et al. (1972) for rainbow trout. Thyroxine levels in fish at 19°C were generally lower than those in fish acclimated to 7°C. Moreover, at 19°C the diet had no effect on thyroxine levels, whereas in the fish acclimated to 7°C, those animals fed a tallow diet had significantly lower plasma thyroxine levels compared with fish fed the soybean oil diet. Evidence indicates that salmonids do not as readily digest the high melting point fats such as tallow as they do oils (NRC-NAS 1973). Thus, the differences in thyroxine levels between the cold-acclimated groups in experiment 3 may reflect the differences in efficiency of lipid digestion and/or absorption.

Narayansingh and Eales (1975a, b) concluded from their studies on brook trout that thyroxine tends to stimulate protein synthesis and the mobilization of free fatty acids. If this is the case in rainbow trout, those fish having a high circulating level of thyroxine might be expected to have rather less carcass fat and rather more carcass protein than fish with lower plasma thyroxine levels. The carcass fat content of both groups of fish held at 7°C (in which were found the highest plasma thyroxine levels), was markedly less than that of fish acclimated to 19°C (see Tables 1, 3). These data may support the conclusions of Narayansingh and Eales (1975b) regarding the role of thyroxine as a lipid mobilizing factor. However, since there were no meaningful differences in the fat content of the two groups of fish acclimated to 7°C despite significant differences in plasma thyroxine levels between these two groups, it would suggest that the differences in carcass fat content are not necessarily directly related to plasma thyroxine levels. There was relatively little difference in carcass protein content in the four groups. If thyroxine is involved in growth, as expressed by protein anabolism, the highest thyroxine levels might be associated with most rapid growth. The data presented here show lowest thyroxine levels in the most rapidly growing fish (i.e. those at 19°C). Thus, the correlation between growth and the action of throxine is not supported by these data. However, the differences in thyroxine levels may be symptomatic

TABLE 3. Effect of diet and temperature on growth and on carcass fat, protein and water content of rainbow trout

Diet	Water temp (°C)	Body wt[a] (*kg*/100 *fish*)	Carcass composition (% *wet wt*)		
			fat	protein	water
Soybean oil	19	6.52	13.49	15.68	68.02
Tallow fat	19	6.20	13.14	15.27	69.88
Soybean oil	7	2.30	9.99	13.70	73.40
Tallow fat	7	2.27	9.05	15.38	73.77

[a]Body weight/100 fish at the commencement of the experiment was 1.02 ± 0.02 kg.

of a difference in the sensitivity (or number) of thyroxine receptor sites in the taget organs of the trout adapted at these extreme temperatures, so that more circulating hormone is required by the cold-adapted trout in order for the target tissue to respond. Certainly, in trout adapted to less extreme temperatures thyroxine is found to stimulate protein anabolism (Narayansingh and Eales 1975a) and thyroxine and tri-iodothyroxine enhance skeletal growth (Qureshi 1976), thus clearly implicating the thyroid in salmonid growth.

An alternative explanation for differences in plasma thyroxine levels in 7 and 19°C acclimated groups may be found from the fact that the fish acclimated to 7°C had grown only very little during the period of the experiment, and their carcass composition was essentially similar to fish of a younger age-group (Cho and Slinger unpublished data). Differences in thyroxine levels of the fish held at the two temperatures may have been related to different "physiological ages" of the two groups, despite their identical "chronological age." A third possibility is that thyroxine may be metabolized more rapidly in fish at a higher temperature. However, measurements of the epithelial cell height in the fish used in experiment 3 provide some evidence that high plasma levels of thyroxine in cold-acclimated fish are due to the higher secretory activity of the thyroid rather than to a reduced usage of the hormone. The histological findings agree closely with those of other authors who found a clear inverse relationship between epithelial cell height and ambient water temperature in salmonid fishes (see review by Swift 1960, also Drury and Eales 1968). The data of plasma thyroxine presented in this report lend support to the hypothesis of these authors that increases in epithelial cell height indicate increased thyroid hormone secretion. Thus, the fish held at 7°C appear to require more thyroxine than fish held at either 15 or 19°C (see Table 1) and may be a reflection of the slower weight gain of the cold-acclimated fish as discussed above (see Table 3).

The data from experiment 2 suggest that the plasma thyroxine levels are not affected by the level of incident light, although the weight-gain rates and feeding behavior were markedly different in the two groups (Cho and Slinger unpublished data). The data from experiment 1 suggest that, at least at the times of sampling used, there was no significant variation in plasma thyroxine concentrations during the light phase of the photoperiod; thus, the time of sampling would not appear to be critical. Similar findings have been reported in mammals (Nye et al. 1976).

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Factors Affecting the Stability of Supplemental Ascorbic Acid in Practical Trout Diets

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HILTON, J. W., C. Y. CHO, AND S. J. SLINGER. 1977. Factors affecting the stability of supplemental ascorbic acid in practical trout diets. J. Fish. Res. Board Can. 34: 683–687.

The stability of L-ascorbic acid in practical trout diets that had been processed by several different methods was investigated. Coated ascorbic acid was more stable to autoclaving than the fine-powdered form, but losses due to water addition, "cold" pelleting, and drying were approximately 90% with or without first autoclaving the diet. Increasing the level of supplemental coated ascorbic acid increased absolute losses but permitted retention of a significant level in the feed for a longer period of time. The shelf life of processed trout diets is dependent on the type of processing, storage temperature, and moisture content of the diet. Laboratory-processed diets stored at room temperature lost all the supplemental ascorbic acid within 6 wk. Commercially processed diets had less processing losses than the laboratory-processed diets, but storage losses were still severe. Freezer-stored diets had minimal losses while diets stored at room temperature were below NRC requirements after 5 wk (steam-pelleted diet) and below this level after 8 wk (extruded diet) when both were initially supplemented with 400 mg/kg feed.

Key Words: ascorbic acid, trout diets, vitamin stability, processing stability, storage stability, vitamin requirements trout

HILTON, J. W., C. Y. CHO, AND S. J. SLINGER. 1977. Factors affecting the stability of supplemental ascorbic acid in practical trout diets. J. Fish. Res. Board Can. 34: 683–687.

Nous avons étudié la stabilité de l'acide L-ascorbique dans des nourritures à truites préparées selon plusieurs méthodes différentes. L'acide ascorbique enrobée est plus stable à l'autoclave que sous forme de poudre fine, mais les pertes résultant de l'addition d'eau, de la formation de boulettes « à froid » et du séchage sont approximativement de 90%, que l'on passe ou non au préalable la nourriture à l'autoclave. Le fait d'augmenter le niveau du supplément d'acide ascorbique enrobée entraîne une augmentation des pertes absolues, mais permet la rétention d'une concentration importante dans la nourriture pendant une plus longue période. La durée de conservation de nourritures à truites préparées dépend du type de traitement, de la température d'entreposage et de la teneur en eau de la nourriture. Des régimes préparés en laboratoire et entreposés à la température ambiante perdent tout le supplément d'acide ascorbique en dedans de 6 sem. Les régimes préparés commercialement subissent moins de perte lors du traitement que les régimes préparés en laboratoire, mais les pertes au cours de l'entreposage sont quand même sérieuses. Les nourritures entreposées dans un congélateur subissent des pertes minimales alors que les nourritures entreposées à la température ambiante sont au-dessous des normes du CNR après 5 sem (régimes en boulettes préparées à la vapeur) et inférieures à ce niveau après 8 sem (nourriture préparée par refoulage) quand l'un et l'autre régime reçoivent au début un supplément de 400 mg/kg de nourriture.

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ASCORBIC acid is an essential vitamin for salmonids (Kitamura et al. 1965; Halver et al. 1969) and must be incorporated as a supplement in practical salmonid diets. This vitamin is unstable under certain conditions (Hay et al. 1967), and Adams (1973) noted that in steam pelleted-crumbled chicken feed, it had poor stability upon processing and during storage. Processing of salmonid feeds requires heat and/or water; losses of the supplemental ascorbic acid would be expected to occur and such feeds may become deficient in this vitamin. Circumstantial evidence suggests that the shelf life of salmonid feeds

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depends, in part, on the ascorbic acid concentration of those feeds after processing and the stability of this vitamin during storage.

The present study was conducted to determine the losses of supplemental ascorbic acid in a practical trout diet (Cho et al. 1974) caused by different types of processing, and to determine the shelf life of processed trout feeds kept under different storage conditions.

Materials and methods — Diet — The practical trout diet used throughout the experiment was similar to diet no. 5 of Cho et al. (1974). Unless otherwise stated the diets were supplemented with 400 mg/kg of 97.5% pure ascorbic acid coated with ethyl cellulose (Hoffmann-LaRoche Ltd., Vaudreuil, Quebec).

Laboratory processing — Type 1. Where indicated the diets were autoclaved (Steam autoclaved model 57-CR, American Sterilizer Co.) at 121°C for 10 min to simulate the heating process used by commercial manufacturers to make steam and extruded pellets. The nonautoclaved and autoclaved diets were then mixed with 25% (V:W) distilled water, separately "cold"-pelleted with the meat grinder attachment on a Braun portable mixer, and dried in a fan-ventillated chamber at room temperature for 24 h. In our experiments 25% water was required to produce a satisfactory pellet and to replace the moisture that is supplied by steam in commercial operations. "Cold" pellets made in the above manner are subjected during pelleting only to the heat of friction generated by extrusion of the feed through the die and this is assumed to be minimal in the presence of 25% water.

Type 2. This processing differed from the former in that the "cold" pellets were produced in a laboratory pellet mill using a 2.4-mm diam die (California Pellet Mill Co., San Francisco, Calif.), in which the temperature of the diet was never higher than 46°C.

Commercial processing — The diet was prepared and processed by a commercial feed manufacturer (Martin Feed Mills Ltd., Elmira, Ontario) into two types of fish feed, steam pellets and extruded pellets; a portion of the mash diet was saved for analysis before processing.

Steam pelleting of the feed was done in a California Hi-flo pellet mill. The feed remained in the conditioning chamber for about 5–6 s where it was heated with steam injected at 1.8 kg/cm² to about 71°C (moisture content of the feed was never greater than 16%) before being pelleted through 4.8-mm diam hole openings in dies 5.1 cm thick with 1.27-cm release. The temperature of the emergent pellets was 75–80°C.

Extrusion pelleting was carried out using a Wenger extruder. Steam was supplied at 7.2 kg/cm², raising the temperature in the conditioning chamber to about 93°C and the moisture to about 20%. The extrusion process required a maximum of 1 min and the feed emerged from the extruder at 135–155°C. The extruded feed was dried in a gas-fired dryer and remained on the conveyor belt for 2 min where the temperature reached 121°C.

Ascorbic acid analysis — The ascorbic acid content of the diets was determined by the 2,6 dichloroindophenol method (AOAC 1970) unless otherwise stated (see Table 5). Triplicate diet samples were homogenized with the extracting solution and centrifuged for 5 min at 3000 rpm. In Experiment 2, the background level of ascorbic acid or interfering substances present in the diet were determined and subtracted from the measured concentrations in the supplemented diets. Due to the types of processing used in Experiments 1 and 3 the measurement of the background level of ascorbic acid was not performed, and therefore the results obtained are not corrected. The percentage recovery of ascorbic acid in the diets by this method was 93.2% ± 2.5.

The 2,4 dinitrophenylhydrazine method (Aeschbacher and Brown 1972) gave recoveries of 95.1% ± 5.0.

Experimental procedure — Experiment 1. The diet (as previously described) and another diet in which coated ascorbic acid was replaced by 400 mg/kg feed of fine powdered ascorbic acid were processed by the type 1 laboratory-processing method. Ascorbic acid analysis of both diets was conducted before and after autoclaving. The ascorbic acid concentration of the coated ascorbic acid diet was also determined after addition of water, after "cold" pelleting, and after drying.

Experiment 2. Seven diets were prepared with graded levels of supplemental coated ascorbic acid from 20 to 1280 mg/kg of diet using geometric increments and a control diet with no supplemental ascorbic acid (see ascorbic acid analysis). The diets were processed by the type 2 laboratory-processing method and ascorbic acid concentration in the diets determined before and immediately after processing and cooling. The diets were stored separately in covered containers in a fish laboratory for 6 wk at room temperature (19–22°C), and their respective ascorbic acid concentrations determined at 1, 4, and 6 wk.

Experiment 3. The ascorbic acid concentration of a diet in three physical forms (mash, steam pelleted, and extruded) processed by the commercial firm was determined immediately after processing. The steam-pelleted and extruded diets were crumbled, and representative samples of these and the mash were stored at room temperature (19–22°C), in a cooler (5°C), and in a freezer (−20°C) for a period of 16 wk; ascorbic acid concentrations were determined at intervals during this storage.

Results — Experiment 1 — The mash diets showed significant losses of supplemental ascorbic acid; the fine-powdered ascorbic acid diet lost 47%, and the diet with coated ascorbic acid lost 26% of that determined in the unprocessed feed (Table 1). The addition of distilled water to the nonautoclaved or autoclaved diets increased the

TABLE 1. Effect of autoclaving on the stability of supplemental ascorbic acid (400 mg/kg feed) in a trout diet.

Diets	Ascorbic acid (*mg/kg feed*)	
	Powdered	Coated
Mash, unprocessed	366±14	399±16
Mash, autoclaved	193±20	295±11
Loss (%)	47	26

losses of ascorbic acid by an additional 71 and 69%, respectively. Cold pelleting resulted in a 10–14% destruction of the ascorbic acid, and drying at room temperature caused a further 3–9% destruction, with cumulative losses of almost 90% of the supplemental ascorbic acid in both diets (Table 2).

Experiment 2 — As the level of supplemental ascorbic acid was increased, losses due to processing increased in absolute terms. Processing completely destroyed up to 40 mg/kg of added ascorbic acid and 74–87% of the higher supplemental levels (Table 3). During the 1st week of storage, destruction of ascorbic acid continued (92–100% losses) and after 6 wk virtually all the supplemental ascorbic acid in the diets had been destroyed.

Experiment 3 — Losses of supplemental ascorbic acid due to processing in a commercial feed mill were also severe; the steam-pelleted diet lost 17% and the extruded diet 33% of the originally determined concentration (Table 4). Ascorbic acid destruction continued at room temperature with the steam-pelleted diet sustaining the greatest loss (96%) after 16 wk. Diets stored in the cooler (5°C) showed substantially smaller losses than at room temperature where the mash lost 1%, steam-pelleted diets 64%, and extruded diets 47% of the amount added after 16 wk. The freezer-stored diets showed virtually no losses of ascorbic acid in the mash and extruded diets, while the steam-pelleted diet had lost 15% after 16 wk (Table 4).

Discussion — These experiments indicate that, because of its instability, great care must be exercised in deciding the type and level of ascorbic acid to include in fish diets, and the time and temperature of storage. Coated ascorbic acid had better stability than the fine-powdered form in the autoclaved fish diets (Table 1), a result that compares favorably with Adams' (1973) observations on a hen diet. Despite the increased stability of coated ascorbic acid, severe losses occurred when the diets were mixed with distilled water, "cold" pelleted, and dried. The fact that both autoclaved and nonautoclaved diets lost about 70% of the supplemental ascorbic acid when water was added (Table 2) suggests that water is a major cause of the destruction. Hay et al. (1967) indicated that alkaline solutions increase the instability of ascorbic acid. The pH of the practical trout diets used in these experiments was 7.8 which may have added to the instability of the vitamin.

Increasing the amount of coated ascorbic acid added to the diet in Experiment 2 resulted in exponential losses, but permitted the retention of significant levels in the feed for a longer period of time. Thus, if it is economically sound, there appears to be some advantage in using substantial margins of safety to offset processing and storage losses. Comparison of the losses from mash vs. processed feeds suggests that processing initiates the destruction of the vitamin which once initiated continues until virtually no supplemental ascorbic acid remains in the diet. Since the 2,6 dichloroindophenol assay measures only the reduced form of ascorbic acid, significant levels of dehydroascorbic acid may still be present in the diets. However, a comparison of the results of the AOAC (1970) method and Aeschbacher and Brown's method (1972), which measures both the reduced and oxidized forms (Table 5), shows that while higher levels of "total" ascorbic acid

TABLE 2. Effects of water, cold pelleting, and drying on the stability of supplemental coated ascorbic acid (400 mg/kg feed) in a trout diet.

Diets	Ascorbic acid (*mg/kg* feed)		Ascorbic acid losses (%)	
	Unprocessed	Autoclaved	Unprocessed	Autoclaved
Mash	399±16	295±12	—	—
Mash, water added	144±21	95±14	71	69
Pelleted, "cold"	76±15	51±15	10	14
Pellets, dried at room temp for 24 h.	40±12	40±21	9	3

TABLE 3. Effects of laboratory processing and room temperature storage on the stability of supplemental coated ascorbic acid in a practical trout diet.

Level of supplementation (*mg/kg*)	Storage period (*wk*)							
	0		1		4		6	
	(*mg ascorbic acid per kg feed* ± SD)							
		% loss		% loss		% loss		% loss
20	0	100	0	100	0	100	0	100
40	0	100	0	100	0	100	0	100
80	16±0	80	0	100	0	100	0	100
160	20±7	87	Trace	100	0	100	0	100
320	84±10	74	21±6	93	Trace	100	0	100
640	102±10	84	49±9	92	15±5	98	0	100
1280	167±23	87	88±10	93	25±10	98	Trace	100

TABLE 4. Effects of commercial processing (mash, steam pelleted, extruded) and storage at different temperatures on the stability of supplemental coated ascorbic acid (400 mg/kg feed) in a practical trout diet.

Storage		Time (*wk*)				
		0	2	5	8	16
		(*mg ascorbic acid per kg feed* ± SD)				
Room temp 21°C	Mash	414±15	389±28	351±40	176±23	95±12
	Steam-pelleted	342±11	211±6	101±10	41±3	14±14
	Extruded	281±6	206±16	136±9	93±5	33±11
Cooler 5°C	Mash	414±15	410±4	379±25	400±18	409±15
	Steam-pelleted	342±11	335±6	307±7	250±15	122±12
	Extruded	281±6	271±4	269±7	250±6	148±16
Freezer −20°C	Mash	414±15	385±23	421±13	397±29	401±14
	Steam-pelleted	342±11	316±8	312±14	310±18	290±14
	Extruded	281±6	269±9	300±6	280±7	295±8

were present, the magnitude of the losses and the exponential nature of the loss curves are not greatly different. Previous experiments in our laboratory (unpublished) have shown that measurement of the ascorbic acid concentration in these same diets after 7 wk of storage by Aeschbacher and Brown's method confirmed the virtually complete destruction of supplemental ascorbic acid in all diets.

Processing and storage losses in commercially manufactured trout diets (Table 4) were not as large as those encountered in the laboratory-processed diets (Experiment 2). This may be due to the reduced amount of water injected in the form of steam in the pelleting and extruding processes. The fact that even the nonprocessed mash diet underwent significant storage losses suggests that oxidation of the ascorbic acid readily occurs at room temperature but has an induction period longer than the processed diets. This destruction is obviously temperature related in that markedly reduced ascorbic acid losses occurred in the diets stored in the cooler or freezer (Table 4). The results again compare favorably with those of Adams (1973). Although the steam-pelleted diet showed less processing destruction of ascorbic acid than the extruded diet, the rate of destruction during storage was greater for the former (Table 4). This may be due to the greater amount of heat and moisture applied to the extruded pellets during processing and to the higher amount of moisture in the steam-pelleted diet (12%) as compared with the extruded pellets (7%) during storage.

TABLE 5. Comparison of ascorbic acid analysis of processed diets (Experiment 2) by the 2,6 dichloroindophinol and the 2,4 dinitrophenylhydrazine methods. *AOAC (1970); **Aeschbacher and Brown (1972).

Level added	2,6 D*	2,4 D**
	(mg/kg/feed)	
20	0	0
40	16±0	20±4
160	20±7	33±6
320	84±10	83±7
640	102±10	169±10
1280	167±23	202±15

Previous work in our laboratory (unpublished) has also shown that extruded feeds were more durable than similar steam-pelleted feeds; the former process may better exclude oxygen from the interior of the particle. It was also found that larger pellets retained ascorbic acid longer than smaller pellets which had the same level of supplementation. On the basis of the NRC (1973) requirement for ascorbic acid in trout diets (100 mg/kg), the shelf life for steam-pelleted diets kept at room temperature is approximately 5 wk and for extruded diets about 8 wk when the diet is supplemented at a level of 400 mg/kg feed with coated ascorbic acid. This does not take into account the leaching losses that occur when the feed is placed in water (about 10% from crumbled pellets in 10 s when 400 mg/kg of ascorbic acid was added to the diet, unpublished data). It is obviously advisable to keep trout diets at colder than room temperatures (ideally in a freezer) until required for feeding. Since this is not feasible under most practical conditions the diets should be mixed at frequent intervals and the margin of safety of supplemental ascorbic acid should be governed by the time the feed will have to be stored.

The effect of water on the stability of ascorbic acid may well have important implications for numerous studies that have used water in the preparation of purified and practical diets to determine the ascorbic acid requirements of fish. If experimental diets have been deficient in ascorbic acid, questions must be raised concerning the validity of other nutrient requirement data obtained with such diets. It is therefore important to determine with considerable accuracy the minimum ascorbic acid requirements of fish under different conditions.

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Survival of American Lobsters (*Homarus americanus*) Stored in a Recirculating, Refrigerated Seawater System

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CORNICK, J. W., AND J. E. STEWART. 1977. Survival of American lobsters (*Homarus americanus*) stored in a recirculating, refrigerated seawater system. J. Fish. Res. Board Can. 34: 688–692.

Conditions required for the extended survival of American lobsters (*Homarus americanus*) in a recirculating, refrigerated seawater system were investigated with a unit incorporating a biologically "conditioned" sand filter with and without activated carbon. Lobsters held at a temperature of 5°C and a density of 50 g/ℓ of water for periods up to 60 days without water change experienced < 10% mortality when activated carbon was incorporated into the filter; without the carbon, mortalities were in excess of 20%. The reduced mortalities with carbon were not related to lower levels of accumulating un-ionized ammonia (toxic form) in the water but were attributed to an inhibition of buildup of acid substances in the water, thereby maintaining pH levels above 7.5.

CORNICK, J. W., AND J. E. STEWART. 1977. Survival of American lobsters (*Homarus americanus*) stored in a recirculating, refrigerated seawater system. J. Fish. Res. Board Can. 34: 688–692.

Nous avons étudié les conditions requises pour la survie prolongée de homards américains (*Homarus americanus*) maintenus dans un système d'eau de mer réfrigérée recirculée, utilisant une unité incorporant un filtre de sable biologiquement « conditionné », avec et sans carbone activé. Les homards maintenus à une température de 5°C et à une densité de 50 g/ℓ d'eau pendant des périodes allant jusqu'à 60 jours sans changement d'eau subissent des mortalités <10% lorsque du carbone activé est incorporé au filtre; sans carbone, les mortalités dépassent 20%. Les mortalités moindres avec le carbone ne sont pas liées à de plus faibles niveaux d'accumulation d'ammoniaque combinée (forme toxique) dans l'eau, mais sont plutôt attribuables à une inhibition de l'accumulation de substances acides dans l'eau, de sorte que le pH est maintenu à des niveaux supérieurs à 7.5.

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AMERICAN lobster (*Homarus americanus*) continues to be the basis for the most valuable individual fishery in Canada. Most of the catch is sold live, requiring storage for periods up to several months in various types of live holding facilities. These facilities usually use ambient temperature sea water, are relatively large, and are restricted to selected coastal areas. Since lobsters are sold in Europe and all over North America, units and procedures for storage in other than coastal areas are required. Such units would, of necessity, require recirculation of the water. Mortality is increased by deteriorating water quality, especially in recirculating systems (Spotte 1970; Hirayama 1974), unless water is changed frequently.

A number of units and operating procedures have been developed to cope with different aspects of these problems (Getchell 1953; Wilder 1953; Wilder and McLeese 1957; Gibson 1959; Thomas 1962; McLeese and Wilder 1964). We have combined a number of these approaches in developing and utilizing a self-contained, refrigerated, recirculating storage unit, based upon Gibson's (1959) concept, to examine factors related to storage densities, development of toxic materials in the water and means to increase the life of the water.

Materials and methods — Each of three storage units consisted of six vertically stacked sliding trays positioned over a refrigerated fiberglass reservoir (Fig. 1). The water in the reservoir was aerated by means of air stones attached to an aircompressor. A pump circulated the water at a constant flow rate through a three compartment filter (containing coarse and medium grades of sand wrapped in nylon netting, and charcoal in certain instances) and thence to the top tray from whence it cascaded through successive trays fitted with water-level control stand-

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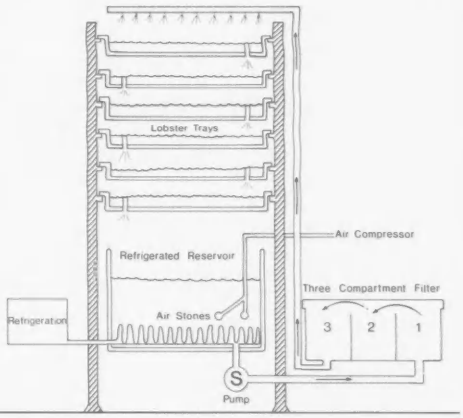

FIG. 1. Schematic diagram of refrigerated lobster storage unit components. The filter normally is positioned in front of and slightly below the refrigerated reservoir but for clarity of diagramming has been drawn to one side.

pipes to the reservoir beneath. The total volume of water in each unit was approximately 450 ℓ.

Healthy, vigorous, freshly captured 450-g lobsters of both sexes, from the Halifax, Nova Scotia, area, were used in the experiment. The claws were immobilized with rubber bands to minimize the effects of fighting. The lobsters were not fed during the experiments.

Stewart et al. (1972) showed that muscle content changes were strictly temperature dependent in starved lobsters held for 3–4 mo. For lobsters starved for 94 days (at 5°C) a 10% loss in muscle content was observed, compared with a 23% loss at 15°C. On the basis of these data, and the fact that lobsters are more resistant to disease (gaffkemia) at the lower temperatures (Stewart et al. 1969), a temperature of 5°C was chosen for all experiments.

Oxygen and nitrate determinations were carried out using Hach (Hach Chemical Co., Ames, Iowa) water test procedures and kits. Total ammonia was determined by the microdiffusion method (Conway 1950), and all values were recalculated and reported as the toxic, un-ionized form using the method of Trussell (1972). The pH of water samples was determined using a Radiometer model PHM-52 meter. Salinity measurements were carried out using hydrometers (Type 4 hydrometer, General Manufacturing Co., New York) calibrated against Copenhagen International Standard sea water.

Experimental and Results — In the first experiment to test storage density, we compared mortalities of lobsters at densities of 75 and 100 g/ℓ of water. In addition, water quality (oxygen, pH, and un-ionized ammonia) was monitored to assess mortality in relation to changing quality. The first two filter compartments were loaded with coarse (rice grade) sand, while the third contained medium grade (Belmont silica #1). The water was not changed during the 24 days of the experiment.

We observed equal weakening and death at both storage densities early in the experiment, so that no conclusions could be drawn about the effect of storage density (Table 1). The percentage of weak and dying lobsters at a density of 100 g/ℓ increased with time until at day 24 approximately 5% of the lobsters were dead and 65% were weak. Wilder and McLeese (1957) recommended water change at 21 days to avoid serious losses of lobsters stored under identical conditions of density and temperature without a filter. The "new" sand filter in this experiment, therefore, did not appear to help water quality. The most significant findings in this experiment were the water quality data. The pH remained unchanged at 8.0 and oxygen and salinity maintained safe levels, at 7.0 mg/ℓ and 31.8‰ respectively. However, the concentration of un-ionized ammonia increased 80-fold to a value of 40.6 nmol/ml, indicating that ammonia toxicity might be responsible for the increased weakness and mortality.

We failed to find published data on the levels of ammonia toxic for lobsters, even though in the un-ionized form it is known to be toxic for many aquatic species (Spotte 1970). The second experiment was to determine the toxicity level of ammonia for the lobster. We tested six groups of five lobsters each at a different concentration of un-ionized ammonia (as NH_4Cl) in static well-aerated sea water at 5°C, and a density of 12.5 g/ℓ. Control lobsters were held in normal sea water under the same conditions. The mean toxicity of ammonia for lobsters was defined as the concentration of un-ionized ammonia causing the death of 50% of the animals in 30 days at a temperature of 5°C. The mean toxicity was approximately 60 nmol/ml (Table 2). This was consistent with the results of Experiment 1, where at 40 nmol/ml 5% of the lobsters were dead and 60% weak after 24 days (although the experimental conditions were not identical). The LT50 (time to 50% death) levels for the two highest concentrations of ammonia tested resulting in 100% deaths were 12 and 22 days, indicating a relatively rapid lethal effect of ammonia.

Our third experiment was designed to determine the efficiency of biological filtration in removing ammonia from the water, and also whether activated carbon (Grade LKC 12/28, Columbia, Union Carbide Ltd., Toronto, Ont.) incorporated into the filters would remove other waste organics and further prolong the quality

TABLE 1. Lobster condition and water quality[a] during storage in recirculating refrigerated sea water.

Storage time (*days*)	Lobsters		Water	
	% Dead	% Weak	Oxygen (*mg/ℓ*)	Un-ionized ammonia (*nmol/ml*)
0	0	0	11.0	0.5
5	1	2	9.0	10.5
12	1	7	9.0	20.1
21	1	16	9.0	36.6
24	5	60	7.0	40.6

[a]Recirculating sea water at 5°C; no water change; sand filter; storage density of 100 g lobster/ℓ of water (approximately 100 lobsters/unit). The pH remained unchanged at 8.0 and the salinity at 31.8‰ during the experiment.

TABLE 2. Ammonia toxicity[a] for lobsters

NH_4Cl concn (*nmol/ml*)	Un-ionized NH_3 (*nmol/ml*)	% Mortality[b]	LT50[c]
40	0.50	0	—
2000	24.4	0	—
3800	46.4	20	—
5000	61.0	60	—
5600	68.3	100	22
8000	97.6	100	12

[a]Temperature — 5°C; storage density — 12.5 g lobster/ℓ water.
[b]At day 30. Groups of five lobsters per concentration.
[c]LT50 — time (days) to 50% mortality.

of the water. Lobsters were stored at a density of 50 g/ℓ of water. In one unit the three filter compartments contained unwashed sand from the previous experiment, while in the other unit the sand in the third compartment was replaced with the activated carbon.

The use of activated carbon in the filter resulted in reduced mortalities (Fig. 2) compared with sand alone, suggesting that organics as well as ammonia had been removed. The ammonia levels (Fig. 3) in both units were considerably lower than in the previous experiment. The unit containing activated carbon had higher ammonia levels during the first 30 days than the one without, although the mortalities in the former case were lower. Ammonia levels remained low throughout the experiment, and mortalities (Fig. 2) continued to increase, paralleled by a reduction in pH (Fig. 4). The addition of activated carbon to the filter slowed the rate of pH reduction and reduced mortalities. Lobsters were successfully stored at a density of 50 g/ℓ of water at 5°C with no water change for periods up to 2 mo with a mortality of $< 10\%$.

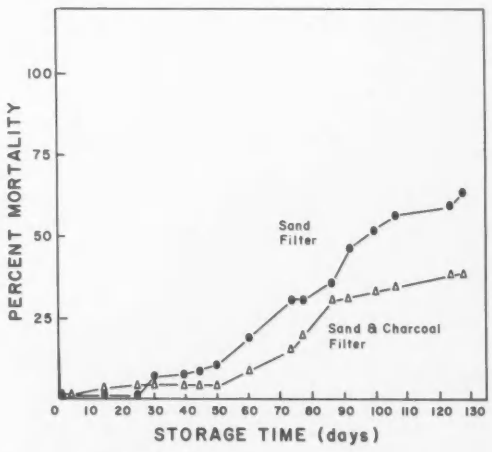

FIG. 2. Mortality rate of lobsters held in a refrigerated (5°C), recirculating seawater unit, at a storage density of 50 g/ℓ of water.

Discussion — When lobsters are held in a recirculating water system at relatively high densities, it is wise not to feed them to avoid fouling of the water (McLeese and Wilder 1964). Lobsters starved for several months, however, lose muscle weight (Stewart et al. 1967; 1972) and are less desirable commercially. We therefore chose a low operating temperature of 5°C for our holding system, to reduce the metabolic rate of the lobsters. As a result they would require less food and oxygen, maintain muscle weight longer, and excrete less toxic material into the water.

Crustaceans, in general, excrete toxic waste products in the form of ammonia and organics into the surrounding water, which become concentrated in a recirculating system. Since physical

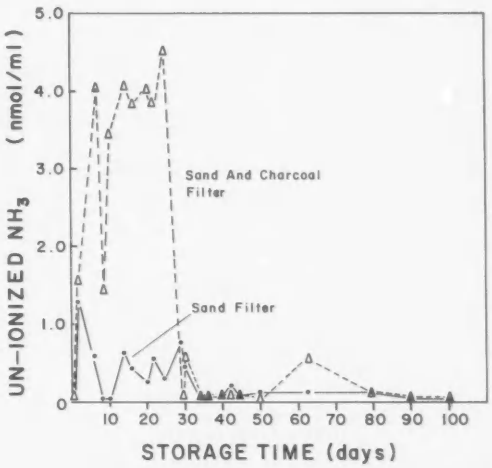

FIG. 3. Un-ionized ammonia levels in refrigerated (5°C), recirculating sea water containing lobsters at a density of 50 g/ℓ of water, with and without carbon filtration.

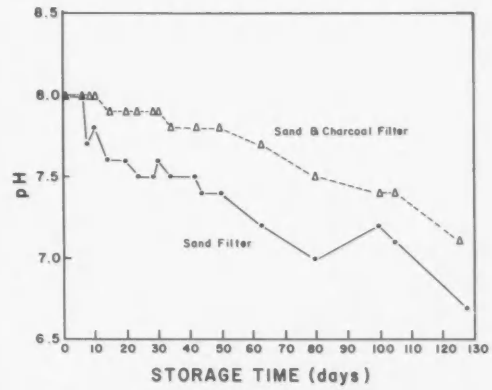

FIG. 4. pH levels in refrigerated (5°C), recirculating sea water containing lobsters at a density of 50 g/ℓ of water, with and without carbon filtration.

stamina and disease resistance of aquatic animals are impaired by ammonia (Burrows 1964), biological filters are important components of recirculating systems. The bacterial components effectively oxidize toxic excretory products such as ammonia and organics to relatively harmless products. In our initial short-term storage experiment the pH, oxygen, and salinity remained stable. The high mortalities appeared to be related to accumulation of ammonia in the water. The biological component of the filter was established in subsequent experiments and ammonia was no longer a problem, but other toxic compounds accumulated. For example, in the third experiment ammonia accumulated initially, but negligible amounts remained after 30 days and mortalities were probably related to the accumulation of other toxic organics. Thus, Binns and Peterson (1969) have shown for the spiny lobster that although the main nitrogenous product is ammonia (90%), excreted through the permeable surfaces such as the gills, some 10% is due to unidentified, complex, nitrogenous compounds in the urine, which probably act as metabolic depressants or pheromones (Karlson and Luscher 1959; Spotte 1970).

The acceptable pH range for marine culture water is 7.5–8.3 (Spotte 1970). Oxidation and the accumulation of toxic organics reduces the pH of the water, which slows down the biological activity of the filter (Atz 1964). In addition, low pH values increase the ratio of the un-ionized, toxic form of ammonia to the ionized, nontoxic form in the water (Downing and Merkens 1955; Warren 1962), making it more detrimental to the lobster. Wilder and McLeese (1957) reported for a storage density of 50 g/ℓ without filter that lobsters lived an average of 53 days and recommended water change at 35 days. Our third experiment with the conditioned filter gave a 20% mortality with the sand filter at 60 days and 50% were still alive at 90 days. The inclusion of carbon, however, reduced the mortality to only 10% after 60 days and 60% were still alive after 130 days. The biological filter apparently had a beneficial effect on lobster survival. We cannot be certain, however, that the difference due to the inclusion of the activated carbon is significant.

These preliminary results suggest that marine biological filters can play an important role in extending the useful life of water in recirculating units. Obviously, more research is required on these filters and their application to large-scale, high density, commercial storage operations. Such studies should include the microbial types involved, the capacity per unit area, and the knowledge required for establishing such filters. These units could fill an important need in areas far removed from sources of natural sea water where artificial seawater mixtures (Wilder 1953) are used, because these mixtures are costly and operators will be looking for maximum life from the water.

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Potential Contribution of Atmospheric Fallout to the Phosphorus Budget of Columbia Lake, Connecticut

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RICH, P. H., AND B. L. PALLOTTI. 1977. Potential contribution of atmospheric fallout to the phosphorus budget of Columbia Lake, Connecticut. J. Fish. Res. Board Can. 34: 692–697.

The Dillon–Rigler Model applied to a heavily "urbanized" lake in New England predicts that less than one half the total input of total phosphorus enters as stream runoff. Of the remaining predicted input, about two thirds may be entering as atmospheric fallout. The balance is suspected to be septic tank seepage and lawn fertilizer runoff. Lake level management dominates the annual pattern of discharge from the watershed and basin and severely restricts the structure of the upper littoral zone. This may have an adverse effect upon the accuracy of the Dillon–Rigler Model.

Key words: phosphorus budget, Columbia Lake, artificial lake, atmospheric fallout

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Le modèle Dillon–Rigler, appliqué à un lac fortement "urbanisé" de la Nouvelle-Angleterre prévoit que moins de la moitié des apports totaux de phosphore proviennent de cours d'eau tributaires. Environ les deux tiers du reste proviendraient de retombées atmosphériques, et l'autre tiers, des infiltrations des fosses septiques et du ruissellement de terrains traités aux fertilisants à gazon. La régulation du niveau du lac agit sur les caractéristiques annuelles du débit du bassin hydrographique, ce qui peut influer négativement sur l'exactitude du modèle.

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A phosphorus budget based upon a model developed by Vollenweider (1969) and modified by Dillon and Rigler (1974) was obtained for Columbia Lake, an urbanized, softwater lake in eastern Connecticut. Lakefront development is concentrated at Columbia, even by New England standards, and occupancy is shifting from seasonal to year-round. Sewage disposal is entirely through septic tank systems which are suspected of contributing nutrients to the lake. Columbia Lake has experienced an increase in summer concentrations of total phosphorus from about 20 to over 50 mg $P \cdot m^{-3}$ in the last 35 yr (Deevey 1940). A recent study (Wells 1973) revealed no significant heterogeneity in the horizontal distribution of phosphorus in the lake. The present study is an attempt to obtain quantitative evidence of nonpoint sources of phosphorus in the lake by using the Dillon–Rigler Model to predict total phosphorus loading on the lake.

Materials and methods — Dillon and Rigler (1974) demonstrated that the concentration of total phosphorus at spring overturn in a number of oligotrophic and mesotrophic lakes in Ontario and Switzerland was related to inputs and outputs of water and phosphorus.

$$[P] = \frac{L(1 - R)}{\bar{z}\rho}$$

[P] = concentration at spring overturn (g $P \cdot m^{-3}$); L = P_{inflow}/lake area (g $P \cdot m^{-2} \cdot yr^{-1}$); $\bar{z}$ = mean depth of lake (m); ρ = lake discharge/lake volume ($m^3 \cdot yr^{-1} \cdot m^{-3}$); $R = 1 - (P_{outflow}/P_{inflow})$. The model was not accurate for very shallow lakes (<1 m) and for eutrophic lakes having phosphorus concentrations $\gtrsim$ 50 mg $P \cdot m^{-3}$. In a subsequent paper (Kirchner and Dillon 1975), R was found to correlate with R_p, a retention estimate derived from the empirical relationship between R and q_s in a number of lakes:

$$R_p = 0.426 \exp(-0.271 q_s) + 0.574 \exp(-0.00949 q_s)$$

where q_s = lake discharge/lake surface area ($m^3 \cdot yr^{-1} \cdot m^{-2}$). Later still, Dillon and Kirchner (1975) found a better correlation between q_s and R_p:

$$R_p = 13.2/(13.2 + q_s)$$

This estimate of R_p will be compared with the R measured at Columbia Lake.

Discharge and total phosphorus concentration were determined weekly at the two inlet streams and one outlet stream at Columbia Lake from April 1975 to April 1976 by the "six-tenths-depth" method (Anon. 1974). Total phosphorus was determined in replicate samples by the perchlorate digestion and molybdate blue method (Strickland and Parsons 1972).

Columbia Lake (Table 1; Fig. 1) is entirely artificial, having been impounded in 1865. A metalimnion appears at the bottom of the epilimnion (ca. 5 m) and extends to the bottom of the lake (Z_{max} = 7.8 m) with no indication of a stagnant hypolimnion. Dissolved oxygen at the bottom is generally well below saturation, but not exhausted. Summer Secchi disk depth averages about 5 m, and *Nitella* sp. colonizes much of the bottom. Lake level is dropped 2–4 m each fall and then raised after ice-out in the spring. Columbia Lake is in the Paleozoic eastern crystalline highlands of Connecticut (Brooks and Deevey 1963) and was reported by Deevey (1940) to have a total phosphorus concentration in the summer of 21 mg $p \cdot m^{-3}$, the highest value for the 20 lakes he studied in the eastern highlands (mean = 10.8 mg $P \cdot m^{-3}$). He reported a Secchi disk depth of 5 m, also, despite the much lower summer [P] at that time. Wells (1973) discovered summer concentrations of total phosphorus in excess of 50 mg $P \cdot m^{-3}$, and [P] = 33 mg $P \cdot m^{-3}$ at spring overturn 1972.

Results — Columbia Lake receives about three quarters of its surface water input and watershed phosphorus from Inlet I (Table 2). Inlet II brings the total surface water input to 7.11×10^6 $m^3 \cdot yr^{-1}$, and the total phosphorus input by this means to 312 kg $P \cdot yr^{-1}$. The rate of phosphorus

TABLE 1. Morphometry and conductivity data for Columbia Lake.

Lake area (A)	1.14 km^2
Watershed area (A_d)	7.43 km^2
Mean depth ($\bar{z}$)	5.1 m
Lake volume (V)	5.77×10^6 m^3
Conductivity	58 μmhos cm^{-3}

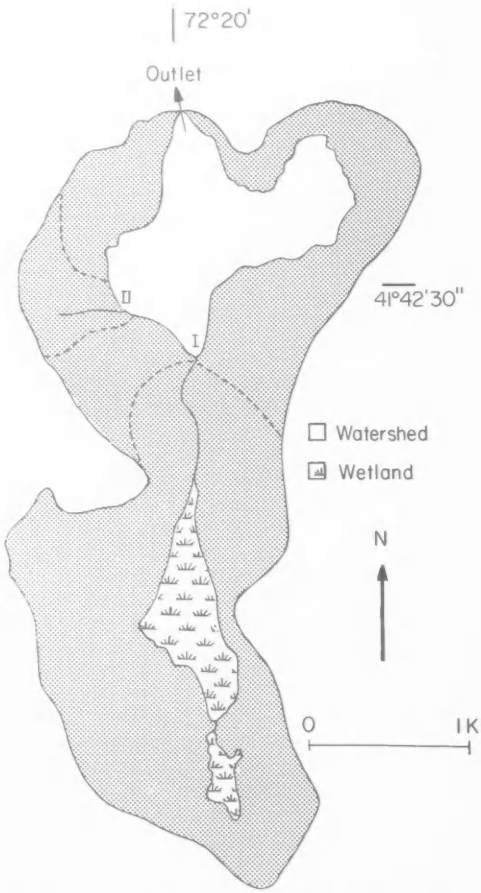

FIG. 1. A watershed map of Columbia Lake, Columbia. Boundaries of stream catchment areas represented by dotted lines.

output by the Columbia Lake watershed (42 kg $P \cdot km^{-2} \cdot yr^{-1}$) is low compared with rates found at most other disturbed forest ecosystems (Likens and Bormann 1974). The estimate of discharge at the outlet is slightly less than the stream inputs, by 6.4%, and is within the error of the methods. Discharge of total phosphorus at the outlet is 240 kg $P \cdot yr^{-1}$, 25% less than the stream inputs. The precision of total phosphorus determinations in the range 8–100 mg $P \cdot m^{-3}$ was ± 9–7%. The error of discharge measurements is unknown.

Despite distinct seasonal variations in the concentration of total phosphorus in discharge from the watershed and lake basin (Fig. 4), the transport of phosphorus (Fig. 3) is strongly correlated with the amount of discharge (Fig. 2), which depends very much upon lake level management. The big discharges of water and phosphorus from the watershed in the winter tend not to be retained in the lake because the dam is open during that period. Total phosphorus accumulates in the

TABLE 2. Estimates of annual discharges of water and total phosphorus from the watershed and basin of Columbia Lake, from April 1975 to April 1976.

	Discharge $(m^3 \cdot yr^{-1}) \times 10^6$	P_{tot} $(kg\ P \cdot yr^{-1})$
Inlet I	5.49	237
Inlet II	1.62	75
$Inlet_{tot}$	7.11	312
Outlet	6.66	235
Balance	.45 (6%)	77 (25%)

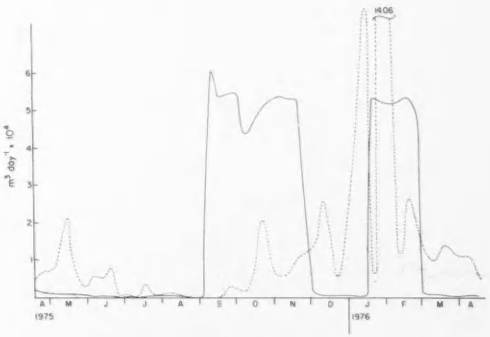

FIG. 2. Discharges of water from the watershed and basin of Columbia Lake between April 1975 and April 1976. Dashed line represents Inlet I; dotted line Inlet II; solid line Outlet.

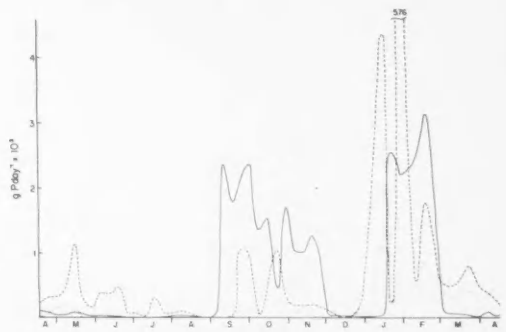

FIG. 3. Discharges of total phosphorus from the watershed and basin of Columbia Lake between April 1975 and April 1976. Dashed line represents Inlet I; dotted line Inlet II; solid line Outlet.

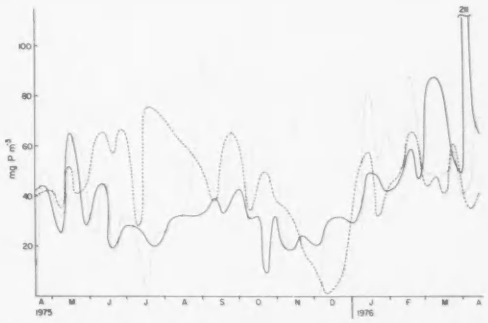

FIG. 4. Concentrations of phosphorus found in the surface discharges of water from the watershed and basin of Columbia Lake. Dashed line represents Inlet I; dotted line Inlet II; solid line Outlet.

lake between March and August when the dam is closed and watershed inputs are moderate and decreasing.

Nonpoint sources of phosphorus are probably less affected by lake level management and more related to chronic and random weather events. The conclusion that lake basin discharge is approximately the same as, or less than, watershed discharge indicates that ground water inputs, i.e. "springs", are not an important source of water for the lake. Thus, septic tank seepage appears not to be aggravated by groundwater dynamics. If anything, subsurface seepage out of the lake is indicated, possibly around and through the earthen dam. Subsurface drainage from the watershed and excess precipitation on the lake surface (less evaporation) could add as much as an additional 25% to the observed 6% excess of surface inputs over surface discharge.

At spring overturn in 1975 [P] was 42 mg $P \cdot m^{-3}$. Solving the Dillon–Rigler Model for [P] using R (Dillon and Rigler 1974), a reasonably accurate estimate is obtained, 35 mg $P \cdot m^{-3}$ (Table 3). Columbia Lake is distinctly eutrophic with respect to phosphorus concentration at spring overturn, apparent phosphorus loading (L = 275 mg $P \cdot mg^{-2}$), and apparent phosphorus loading adjusted for water renewal rate (ρ = 1.2) (Vollenweider 1968; Kerekes 1973, 1975). Solving the model for [P] using Dillon and Kirchner's $R_p = 13.2/(13.2 + q_s)$ produces a severe underestimate, 14.5 mg $P \cdot m^{-3}$. The estimate of phosphorus removal by the lake based upon the areal water load (R_p) is more than twice the estimate based upon actual watershed and lake discharges (R), causing the underestimate of [P]. In other words, [P] at Columbia Lake is accurately predicted by watershed inputs, but the lake basin apparently retains less incoming phosphorus than expected based upon the annual amount of water that flows over a unit area of the basin.

TABLE 3. Flushing rate (ρ), measured total phosphorus loading rate (L), measured total phosphorus retention coefficient (R), R estimated by the areal water load (R_p), and [P] predicted by ($L(1 - R)/\bar{z}$) for Columbia Lake.

ρ	1.2 $m^3 \cdot yr^{-1} \cdot m^{-3}$
L	275 mg $P \cdot m^{-2} \cdot yr^{-1}$
R	0.25
$R_p = 13.2/(13/2 + q_s)$	0.69
$L(1 - R)/\bar{z}\rho$	35 mg $P \cdot m^{-3}$
$L(1 - R_p)/\bar{z}\rho$	15 mg $P \cdot m^{-3}$

Discussion — The agreement between the actual [P] at Columbia Lake at spring overturn and [P] predicted by the unmodified Dillon–Rigler Model when only surface water discharges are considered appears suspicious. Important atmospheric and nonpoint source inputs of phosphorus are expected at Columbia. Further, the discrepancy between the actual (R) and predicted (R_p) estimates of phosphorus retention is consistent with the hypothesis that surface water discharge is not the only important source of phosphorus. The concentration of phosphorus could be "correctly" estimated when using R in the model despite "unknown" sources of phosphorus input. The loading factor, $L = P_{inflow}$/lake area, would be underestimated by an amount proportional to unknown input(s) of total phosphorus. Simultaneously, the retention coefficient, $R = 1 - (P_{outflow}/P_{inflow})$, would be underestimated because unknown and nonpoint sources of phosphorus appearing in the outflow would inflate the value of the ratio $P_{outflow}/P_{inflow}$. These errors would tend to cancel in the numerator of the model, $L(1 - R)$.

Using [P] = 35 mg $P \cdot m^{-3}$ and $R_p = 0.69$ solving the Dillon–Rigler Model for L gives:

$$L = \frac{[P]\bar{z}}{(1 - R_p)} = 580 \text{ mg P m}^{-2} \cdot \text{yr}^{-1}$$

This prediction of L increases the estimate of total phosphorus entering Columbia Lake to 660 kg $P \cdot yr^{-1}$ and requires an input of phosphorus from unknown and nonpoint sources of 350 kg $P \cdot yr^{-1}$.

The apparent discrepancy in the water budget, which suggests that as much as 30% more water enters the lake than leaves, does not appear to offer an explanation of the underestimate of R. Balancing the budget by decreasing input or increasing discharge would tend to make the ex-

pression

$$R = 1 - (P_{outflow}/P_{inflow})$$

even smaller.

Non-point sources of phosphorus — Sources of phosphorus not measured in the stream inputs would include atmospheric fallout (wet or dry) directly on the lake surface, immediate runoff of atmospheric fallout from lakefront property and storm drains, septic tank seepage, runoff of fertilizers used on lakefront lawns and gardens, etc. Connecticut is on the Boston–Washington corridor and downwind of the New York–New Jersey industrial complex, so atmospheric fallout is expected to be very high. Using a high estimate of phosphorus fallout, 100 mg $P \cdot m^{-2} \cdot yr^{-1}$ (Wetzel 1975), 115 kg $P \cdot yr^{-1}$ can be expected to fall directly on Columbia Lake. Assuming that Columbia Lake receives storm drainage from a lakefront area roughly equivalent to its own surface area, immediate drainage can be estimated to be about equal to direct atmospheric inputs. Adding these estimates to stream inputs leaves a unaccounted balance of about 20% of the total predicted input of phosphorus (Table 4.). This balance, 120 $kg \cdot yr^{-1}$, is attributed primarily to lawn fertilization and septic tank seepage.

Alternatives to the hypothesis that discrepancies between actual and predicted values of $[L]$ and R are caused by anthropogenic inputs of total phosphorus appear less likely. An apparently low R might be caused by the presence of an extremely refractory, biologically inactive form of P which does not sediment but appears in the determination of total phosphorus. Such a compound would have to be found in Columbia Lake, but not in lakes in Ontario and Switzerland (Dillon and Rigler 1974) and Newfoundland and Nova Scotia (Kerekes 1973, 1975). An apparently low R might also be caused by phosphorus released from the sediments of Columbia lake, possibly a consequence of incomplete thermal stratification and the fact that Columbia lake is artificial and overlies a terrestrially derived soil. Based upon the estimated input balance of 120 kg $P \cdot yr^{-1}$, 100+ mg $P \cdot m^{-2} \cdot yr^{-1}$ would be required from the lake sediments. Terrestrial soils of medium fertility are generally capable of exporting only 50 mg $P \cdot m^{-2} \cdot yr^{-1}$ (Wetzel 1975), and this would be expected to be decreasing, not increasing, after a total of 110 yr submergence.

TABLE 4. Inputs of total phosphorus at Columbia Lake based upon phosphorus loading predicted by Dillon–Rigler Model and 100 mg $P \cdot m^{-2} \cdot yr^{-1}$ atmospheric fallout.

Source	P input ($kg \cdot yr^{-1}$)
Stream discharge (measured)	310
Atmospheric fallout (direct) (estimated)	115
Immediate runoff (estimated)	115
Predicted total input	660
Balance (septic tank seepage, lawn fertilization, etc.)	120

Management of lake level — The annual cycle of lake level management may have a significant impact upon the validity of the Dillon–Rigler Model for Columbia Lake. Exposure of the upper littoral zone during the winter has a catastrophic effect upon many organisms normally found there. The shallow waters of Columbia Lake are rather barren of aquatic macrophytes, molluscs, and most other invertebrates, although epilithic and epipelic algae are abundant during high water. Thus, the role of nutrient interception and mediation associated with the littoral zone (Wetzel 1975) may be impaired at Columbia Lake, and this may change relationships among the parameters of the model.

At the time of ice-out, 1976, extremely high [P], i.e. 80+ mg $P \cdot m^{-3}$, was found in the outflow (Fig. 4). This is attributed to the thawing of winter precipitation and runoff from the exposed lake bottom. During the period between ice-out and the closing of the dam, inflows, including very minor ones from storm drains, eroded deep gullies in the soft sediments exposed between outfall and water's edge.

Effects of water-level management upon the model would appear to increase phosphorus outflow by reducing the phosphorus storage capacity of the littoral zone and by mobilizing sediments during the thaw. Yet, the perennial *Nitella* sp. in the deep water may replace littoral annuals as storage for phosphorus, given the lack of summer stratification at Columbia Lake, and this may prevent a spring flush of phosphorus from dead and decaying littoral plants into the water. Further, the runoff and transport of phosphorus from the shallow littoral to greater depths may remove significant amounts of phosphorus from a zone of uptake and translocation by rooted macrophytes to a zone of sedimentation and burial.

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Currents in Johnstone Strait, British Columbia: Supplemental Data on the Vancouver Island Side

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THOMSON, R. E. 1977. Currents in Johnstone Strait, British Columbia: supplemental data on the Vancouver Island side. J. Fish. Res. Board Can. 34: 697–703.

Records from a current meter recently found adrift after 3 yr have been used to complete previously published cross sections of mean flow and tidal currents in Johnstone Strait. The new plots emphasize the cross-channel asymmetry of the estuarine circulation and the large phase lag of the upper layer current relative to the bottom currents for the diurnal (K_1) tidal constituent. A recalculation of the net volume transport shows that its magnitude and direction remain undeterminable to the order of $\pm 10^3\ m^3 \cdot s^{-1}$.

Key words: tidal currents, residual currents, estuarine circulation, Johnstone Strait

THOMSON, R. E. 1977. Currents in Johnstone Strait, British Columbia: supplemental data on the Vancouver Island side. J. Fish. Res. Board Can. 34: 697–703.

Les données contenues dans un courantomètre trouvé à la dérive après 3 a ont servi à compléter des tracés déjà publiés du débit moyen et des courants de marée dans le détroit de Johnstone. Les nouveaux tracés soulignent l'assymétrie de la circulation dans l'estuaire du détroit ainsi que le gros décalage de phase du courant de la couche supérieure par rapport aux

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courants du fond pour le constituant (K_1) des marées diurnes. Un nouveau calcul du volume net d'eau transporté indique que sa grandeur et sa direction restent indéterminables avec plus de précision que $\pm 10^3$ m$^3 \cdot$s^{-1}.

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FROM February to June 1973, the Canadian Hydrographic Service maintained current meter moorings at five locations across the central portion of Johnstone Strait. Velocity, temperature, and conductivity data from four stations were retrieved at the termination of each of two 60-day recording intervals and certain aspects of that data published (Thomson 1976). Unfortunately the fifth mooring, located in about 100 m of water 0.62 km south–southwest of Station 4, was never recovered. As a consequence, the cross-channel plots of Thomson (1976) contained a substantial gap near the Vancouver Island shore. In June 1976, however, the near-surface current meter from Station 5 was found adrift approximately 30 km west of the original experimental site, its magnetic tape readable despite the almost fully corroded condition of the instrument case. This somewhat fortuitous discovery has made it possible to present a more complete picture of the tidal and estuarine current structure across the strait.

Materials and methods — At the time of deployment on February 21, 1973, Station 5 consisted of a bottom-moored, ¼-in. (6.3-mm) stainless steel line with subsurface floatation in about 100 m of water. Aanderaa model-4 current meters recording speed, direction and temperature every 15 min on ¼-in. magnetic tape were positioned on the line at depths of 15 and 50 m. Recovery of the mooring was to have been facilitated via a plastic surface buoy attached to the upper meter by ¾-in. (18.9-mm) polypropylene rope.

When the upper instrument and buoy were found after 3 yr in the Johnstone Strait region, only the plastic parts of the meter were salvageable. The magnetic tape was encrusted with barnacles but after cleansing in fresh water and alcohol appeared to have suffered little physical damage. Of the nearly 9000 records, however, approximately 1000 had obviously incorrect directional values mostly in the form of isolated "spikes" having a digital value of "0" on the magnetic tape. The distribution of these zeroes was not totally random but showed an apparent reluctance of the meter to record in the southwest quadrant, suggesting that it functioned with a faulty contact in the compass unit. Corrections to these erroneous readings have been made in the raw data by linearly interpolating between values before and after the appearance of a spike. Nonzero spikes occurred infrequently and were corrected with the further assumption that the current is predominantly east–west with semidiurnal frequency.

Results — Instrument depth and mean currents — The steady seaward (westward) drift during the first part of the progressive vector diagram (Fig. 1) is consistent with the upper layer mean flow described in Thomson (1976). This indicates that the mooring remained in its initial position for at least 25 days (from Julian day 52–77) before it slumped or was dragged by a passing vessel into deeper water near its original anchorage. This portion of the record is considered as representative of currents at 15 m near the Vancouver Island shore. During the second portion, on the other hand, there is a distinct change in the character of the record as the mooring slumped toward midchannel. (This portion consists of two separate intervals; the first extends from Julian day 77 to around day 100 and the second from day 100 to the end of the record. Since currents during the former period are somewhat erratic and show nontidal direction reversals, they have been omitted in the subsequent analysis.) After its relocation down the steep side of the channel, the upper current meter remained in sufficiently shallow water to still record a net seaward flow. However, its absolute depth cannot be determined without further information. Projection of the known depth of the zero mean-flow level at Stations 3 and 4 over to Station 5 (Fig. 2) suggests that the current meter was shallower than 80 m. In particular, plotting the mean eastward component obtained during this period on the line joining the 80-m null-depth with the mean eastward value at 15 m depth yields a depth of roughly 55 m for the relocated upper meter. Some support for this calculation is derived from the fact that the mean vertical shear at Station 5 of $1.47 \times 10^{-3} \cdot$s^{-1} is essentially equal to that measured at all other locations. (The mean northward currents $\bar{v}$ at Station 5 are small and below the threshold level of 1.5 cm$\cdot$s^{-1} for the Aanderaa's; at 15 m, $\bar{v} = +0.3$ cm$\cdot$s^{-1} and at 55 m, $\bar{v} = -0.9$ cm$\cdot$s^{-1}.)

Subsequent to the mooring attaining its new location, salt water erroded the stainless steel wire to the point where the upper meter and attached surface buoy could break free. Growth on the instrument and fiber rope was appreciable, indicating that they may have been further exposed to a period of euphotic conditions on a beach or shoal.

In Fig. 3, the strike of the channel has been

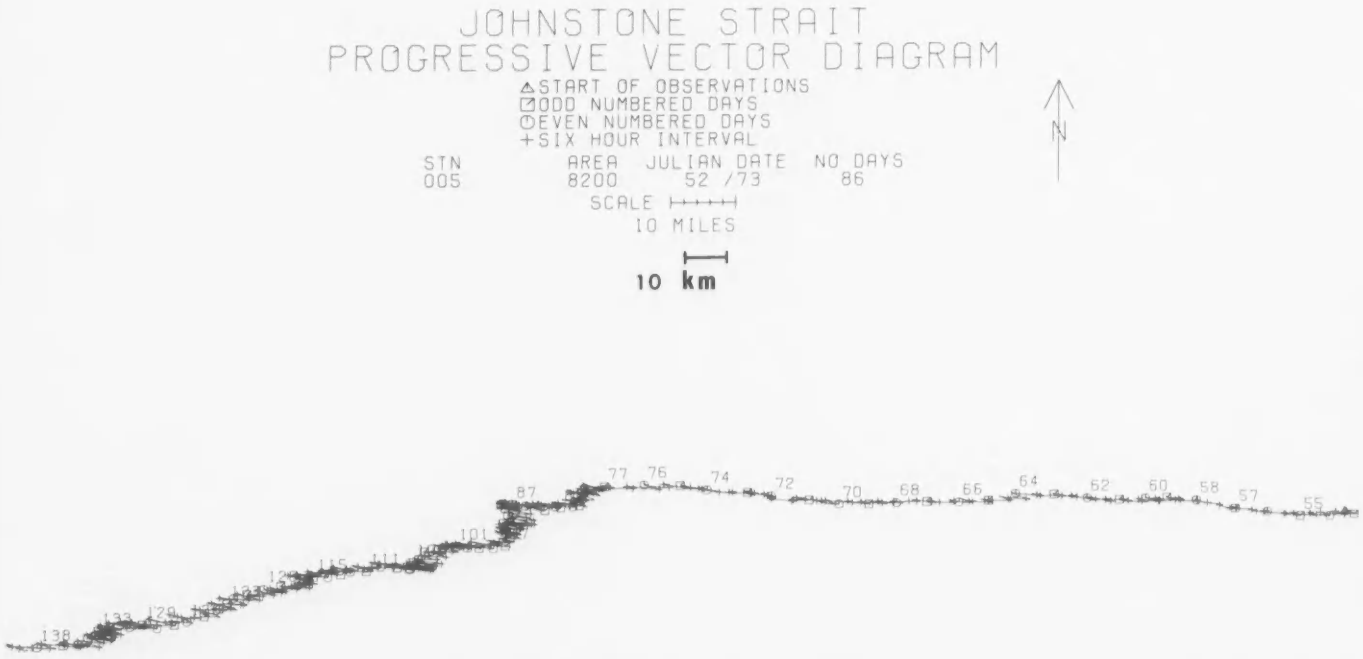

FIG. 1. Progressive vector diagram for the upper current meter at Station 5. The record begins at 1500 on February 21, 1973 (Julian day 52) and ends at 1600 on May 18, 1973 (Julian day 138). The instrument depth is assumed to have been 15 m up to day 77 and 55 m thereafter.

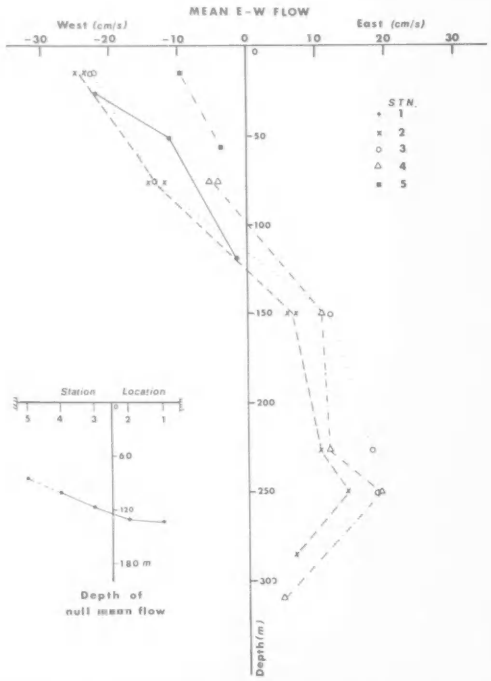

FIG. 2. The mean east–west component of the current vs. depth. Averages have been taken over two separate deployment periods and for depths above 150 m may therefore have two values. The insert shows the "observed" depth of the zero-mean flow level at Stations 1 through 4 and the laterally interpolated zero-mean flow level for Station 5 (~80 m). (This figure also corrects the curve for Station 1 in Thomson 1976; c.f. Fig. 5A.)

taken as −10° to the eastward direction and where there are two separate recording intervals for a given depth the average value has been used. As the mean values listed in this figure indicate, the latter are generally within 10%. (Record lengths in days are given in Fig. 2 of Thomson 1976.)

An estimate of the net transport through Johnstone Strait can be made through a cross-sectional integration of the currents in Fig. 3. Based on linear interpolation of the speeds between adjacent contours and assuming no-slip at the boundaries, the seaward transport (U_u) in the upper layer was −33.2 (±10%) × 10^3 $m^3 \cdot s^{-1}$ and the inward transport (U_l) in the lower layer was +35.2 (±10%) × 10^3 $m^3 \cdot s^{-1}$, giving a net inward transport of +2 × 10^3 $m^3 \cdot s^{-1}$. This result is unreliable however because of the large errors involved. For example, a uniform vertical lowering of the depth of the null-speed level by only 8 m in Fig. 3 would decrease the net transport to zero. The best estimate therefore is that the volume transport through the central portion of Johnstone Strait is of the order of ±10^3 $m^3 \cdot s^{-1}$.

Tidal currents — In agreement with the harmonic analysis of Thomson (1976), tidal currents at Station 5 are predominantly semidiurnal. In particular, the ratio of the major diurnal constituents K_1 and O_1 to the major semidiurnal constituents M_2 and S_2 was found to be $(K_1 + O_1)/(M_2 + S_2) = 0.148$ at 15 m depth with a slight increase to 0.268 at 55 m depth. Moreover, these values are consistent with the relatively low values (<0.3) obtained on the opposite side of the channel at Station 1. At intermediate locations the ratio exceeds 0.3.

Figure 4 completes cross-channel plots of the magnitude and Greenwich phase lags for the major semidiurnal (M_2) and diurnal (K_1) constituents, respectively (c.f. Thomson 1976, 9a, b). The M_2 constituent is now seen to have cross-channel symmetry with respect to phase in which the currents in the central part of the strait lead those at either shore by approximately 30° (~1 h). Again we find maximum speeds at this frequency at depths of around 250 m near midchannel but also an indication of relatively strong flow at 50 m near the Vancouver Island shore.

In the case of the diurnal (K_1) constituent, the tidal currents near the channel floor and sides lead those near the surface by 50–75° (3.3–5 h).

Residual motions — The current meter record at Station 5 was low-pass filtered to remove the daily tidal fluctuations (as in Thomson 1976). A quasi-fortnightly modulation of the current speeds was again evident while the variation in the residual flow was apparently more pronounced at a depth of 55 m (Fig. 5B) than at 15 m (Fig. 5A) in contrast to previous findings. In particular, the 15 $cm \cdot s^{-1}$ range of this flow at 55 m depth was roughly twice that at the near-surface instrument. There was moreover no definite surface wind effect during the storm of February 25–28 (Julian days 56–59), although the somewhat more intense easterlies of March 17–21 (days 77–81) and appear to alter to the surface current (c.f. Thomson 1976, Table 1). It is perhaps not accidential that the beginning of the latter storm (mean wind speed 9.5 $m \cdot s^{-1}$) coincided with the time of marked change in the nature of the current meter record (Fig. 1) and with the commencement of mooring slump.

Finally, the temperatures measured by thermistors attached to the current meter showed a net increase of 0.20 deg C over the 27-day period at 15 m and increase of 0.50 deg C over the 69-day period at 55 m. At the latter depth, large flood currents were generally accompanied by

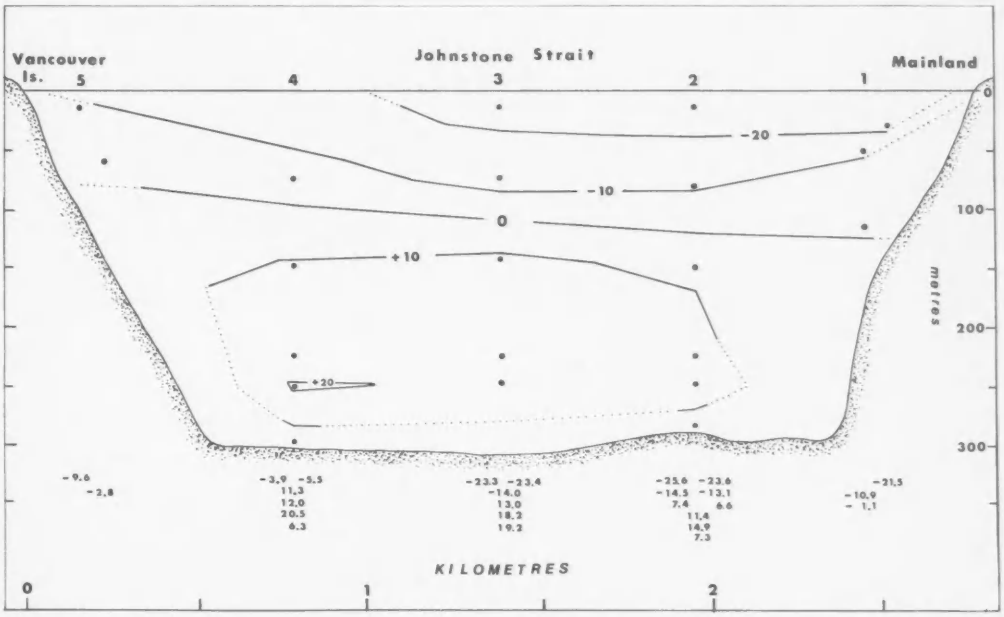

FIG. 3. The time averaged along-the-channel current structure across the central portion of Johnstone Strait. Speeds are in centimetres per second, negative values indicate a seaward (westward) flow and positive values an inward (eastward) flow. The channel is oriented at −10° to the eastward direction. Observed values are listed below each mooring location. Dotted lines represent uncertain interpolated values.

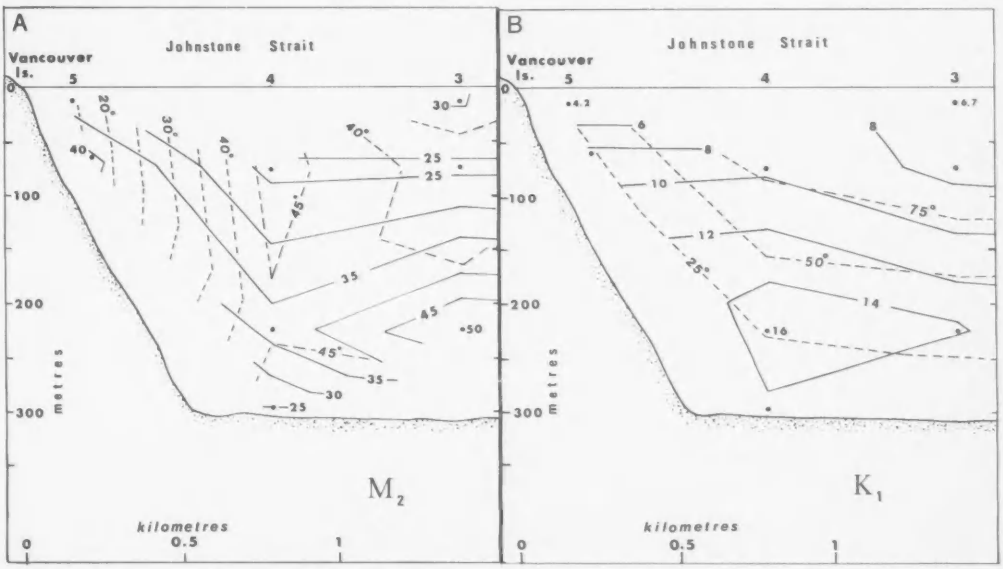

FIG. 4. A, Plot showing the magnitude of the M_2 tidal constituent in cm·s^{-1} (solid line) and its Greenwich phase lags in degrees (dashed line) across the southern half of the strait for the period February 20, to April 10, 1973. Where there is any possible ambiguity in speeds, actual values have been placed at the appropriate depths. B, Plot showing the corresponding magnitude and phase lag of the K_1 tidal constituent for the same period as 4A. (Complete Thomson 1976, Fig. 9a, b).

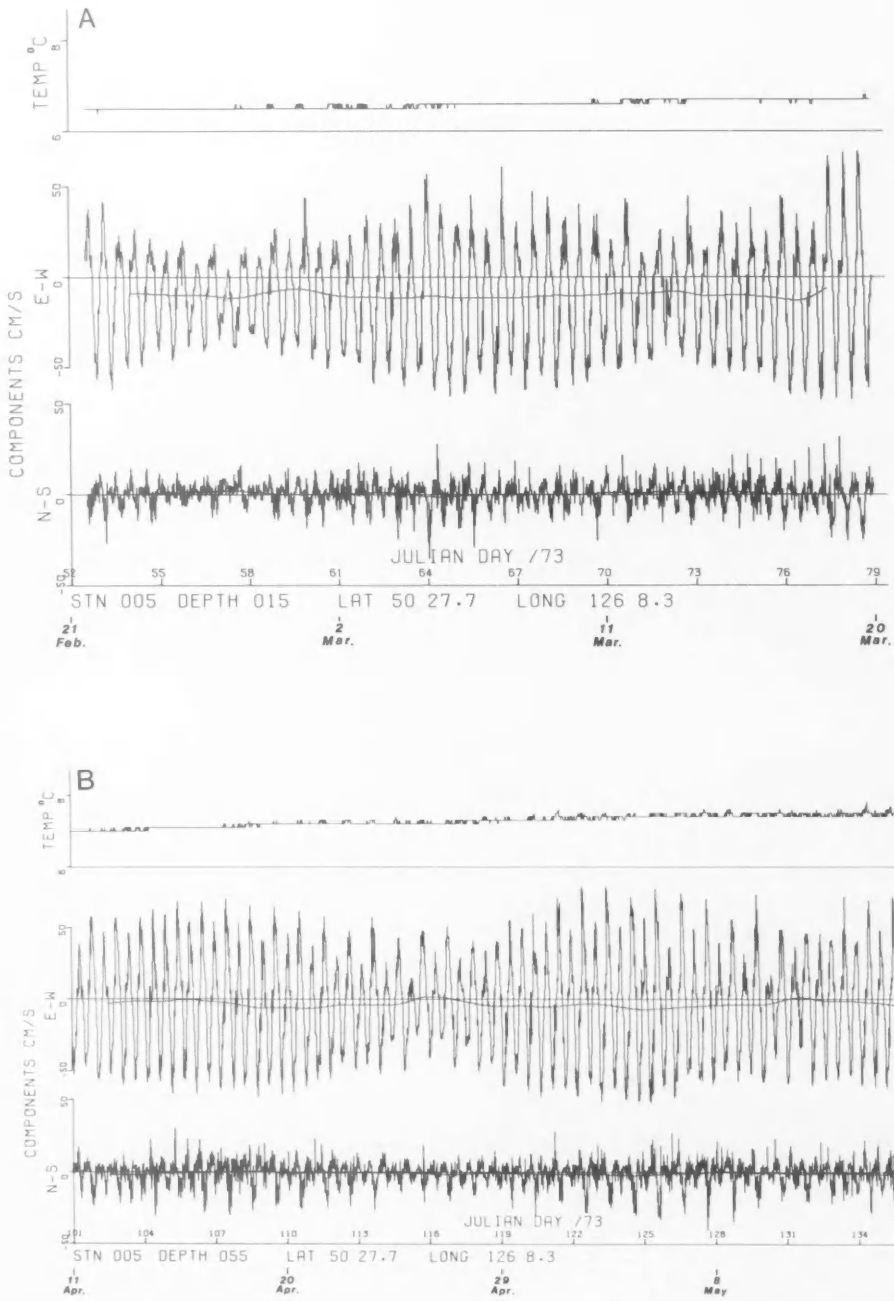

FIG. 5. A, Plot of the east–west and north–south current components and associated residual current at a depth of 15 m at Station 5. Temperature is also shown. B, Corresponding plot to A for the current speeds at a depth of 55 m at Station 5.

small positive fluctuations in water temperature, in agreement with previous findings for the deeper levels at Stations 1–4. The above trends in temperature were comparable to those at other locations across the channel (Thomson 1976).

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Copepod Instar Survival and Predation by Zooplankton

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CONFER, J. L., AND J. M. COOLEY. 1977. Copepod instar survival and predation by zooplankton. J. Fish. Res. Board Can. 34: 703–706.

The survivorship of each instar of three cohorts of *Leptodiaptomus minutus* from Bluff Lake, Nova Scotia, is presented. High naupliar mortality but low copepodite mortality occurred. The previously reported values for the predation rate and size selection of prey by omnivorous zooplankton are compared with the observed mortality rates for the different sizes of instars. We estimate that predation by the omnivorous zooplankton could account for most of the naupliar mortality. Our analysis supports Dodson's hypothesis that size-selective predation by zooplankton can be a significant factor determining the proportion of a cohort that reaches maturity.

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La survie de chaque stade larvaire de trois groupes de *Leptodiaptomus minutus* provenant du lac Bluff en Nouvelle-Écosse est étudiée. La mortalité des nauplius était élevée tandis que celle des copépodites était faible. Les données déjà publiées sur le taux de prédation et sur la sélection des proies selon la taille par le zooplancton omnivore sont comparées aux taux observés de mortalité chez les larves de différentes tailles. Nous estimons que la prédation par le zooplancton omnivore pourrait être la principale cause de mortalité des nauplius. Notre analyse renforce l'hypothèse de Dodson que la prédation sélective par le zooplancton et qui s'exerce en fonction de la taille peut être un important facteur déterminant de la proportion des larves d'un groupe qui arrivent à maturité.

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ZOOPLANKTON communities are often dominated by large species when vertebrate predation is low. Brooks and Dodson (1965) hypothesized that the large species are able to out-compete the small species for the limiting food supply. Efforts to verify this hypothesis have produced inconclusive results, and Dodson (1974) has proposed a new hypothesis explaining this common observation. He has proposed that selective predation on the small zooplankton by the large predacious zooplankton is the principal reason that small species are reduced or eliminated. Dodson's supportive evidence was derived from studies of six alpine ponds. While the data appear convincing as applied to these unusual ponds they do not establish that the hypothesis is generally applicable to many kinds of aquatic systems.

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Herein we report additional information, obtained from a more typical lake community, that supports Dodson's hypothesis. The survivorship of each instar of each cohort of a small herbivorous copepod in Bluff Lake, Nova Scotia, was analyzed by Cooley (1973). The abundance of the two most numerous species of predacious zooplankton was also determined. We use published predation rates for similar predators on specific sizes or stages of prey to estimate the fraction of the observed mortality of the herbivore accountable to the predacious zooplankton in this lake.

Materials and methods — The survivorship of each instar for three cohorts of *Leptodiaptomus minutus* in Bluff Lake was presented in detail by Cooley (1973). Bluff Lake with an area of 4.8 ha and a maximum depth of 7 m has already been described by Hayes et al. (1952). The population data obtained by Cooley is the basis for the calculations we present. Quantitative samples of the limnetic zooplankton were taken from October 17, 1970 to May 19, 1972 with a transparent trap (Schindler 1969) from a small boat anchored at various stations on the lake. Samples were collected every 4th or 5th day from May through early fall, every 6–7th day in late fall, and once or twice a month through the ice. On most days approximately 40 samples were taken at five fixed stations but occasionally fewer stations were sampled during the winter of 1970–71, especially when the ice was thin. On all days the entire water column at 0.5-m intervals was sampled to within 0.5 m of the bottom of each station. In all a total of over 2300 samples was collected. From these samples the abundance of some copedodite stages of the major predacious zooplankters in the lake, *Mesocyclops edax* and *Epischura nordenskioldi* was also determined.

Results — Figure 1 shows the survivorship of three cohorts of *L. minutus* in Bluff Lake and the abundance of the two major zooplanktonic predators (Cooley 1973). Survivorship was calculated using the method of Rigler and Cooley (1974). Their method allows one to estimate the mean number of animals surviving in each instar of a cohort. In Fig. 1 the initial abundance of each cohort has been prorated to 1000. Each point in the upper panel is the prorated proportion of a cohort remaining at the time of mean abundance for each instar. The slope of the lines connecting any two successive instars is proportional to the death rate. Survivorship through the six naupliar instars is low for all three cohorts: 18, 5, and 12%, respectively. Only N1 through N3 of cohort 1, which appeared before any copepodites of any predacious zooplankton, have high survival rates. Survivorship of the larger copepodite instars is much greater than the survivorship of the nauplii. This is particularly evident for the nauplii of cohort 2 compared with the concurrent copepodites of cohort 1. The much higher percentage of copepodites that survive between successive instars is further emphasized when one considers that the duration of copepodite instars is much longer than the duration of naupliar instars. A similar pattern of high mortality of nauplii has been reported for *Skistodiaptomus oregonesis* in Teapot Lake by Rigler and Cooley (1974), and for *Diaptomus clavipes* by Ghers and Robertson (1975).

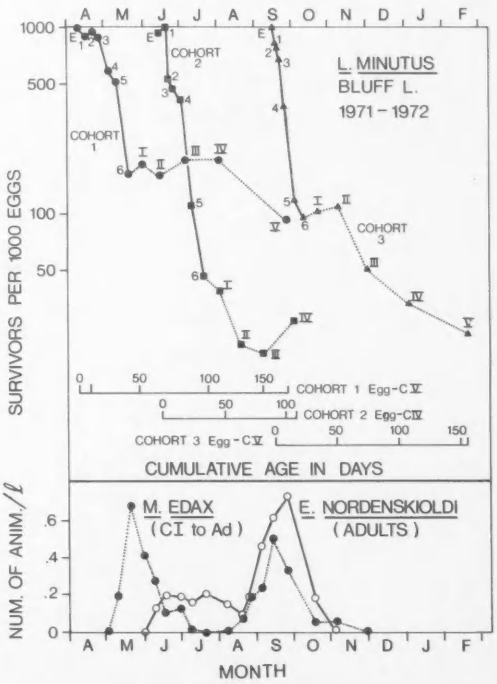

FIG. 1. (*Top*) The survivorship of three cohorts of *Leptodiaptomus minutus* in Bluff Lake, N.S., 1971–72. Naupliar instars are here designated with arabic numerals while copepodite instars are designated with a capital C and the appropriate roman numeral. The estimates of the egg abundance producing each of the three cohorts are respectively, 4384, 2933, and 3490 $\times$ $10^2/m^2$. Mortality estimates could not be made with confidence past the CIV instar in cohort 2 due to very low abundances. (*Bottom*) The abundance of the omnivores *Mesocyclops edax* and *Epischura nordenskioldi* in Bluff Lake during the same time.

Cooley (1973) was primarily concerned with the herbivorous copepod, but fortunately additional data was obtained on other zooplankton in the samples. Of all the other species the two most

abundant predators were *E. nordenskioldi* and *M. edax*. Their absolute abundance is shown in the lower panel of Fig. 1.

Discussion — In the following we summarize the available evidence relative to size selective predation by copepods and the laboratory studies of the rate of such predation. From Cooley's (1973) data on the naupliar mortality, and his data on the abundance of the predacious zooplankton, we have calculated the rate of predation per predator that would be necessary to account fully for the naupliar deaths. This rate, which would account for all naupliar mortality, is compared with the rate of predation observed in laboratory studies.

Confer and Blades (1975) have summarized eight studies of the comparative predation rates of omnivorous zooplankton on different sizes or species of prey. Out of 39 predator–prey pairs considered in the eight studies, the 19 prey species or size categories eaten most frequently were all smaller than the predator. Usually the selected prey were nauplii and early copepodites smaller than 1.0 mm. Anderson (1970) found with his extensive data that the rate of predation by *Hesperodiaptomus arcticus*, *H. shoshone*, and *H. nevadensis* increased significantly as the size of the prey decreased. Confer and Blades (1975) observed almost a ten-fold greater rate of predation by *E. lacustris* on *L. sicilis* nauplii and early copepodites (0.18–0.54 mm) than on adults (0.81–0.98 mm). Confer (1971) found greater predation by *M. edax* on CIII stages of *Arctodiaptomus floridanus* than on adults, but did not investigate naupliar predation.

In nearly all of the experiments summarized by Confer and Blades the predators were not offered two or more prey types simultaneously. Only Dodson's (1974) data and a small part of Anderson's (1970) data were obtained by offering the predator a choice of prey. Consequently, only this small portion of the data convincingly documents size selection. This convincing data plus the uniformity of results from the many experiments of weaker design are highly suggestive that predacious zooplankton usually select for small prey.

The validity of Dodson's hypothesis requires not only that the predacious zooplankton exert a size-selective predation, but also that the rate of predation by each predator and the abundance of the predators be sufficient to significantly effect the abundance of small herbivores. Rates of predation by omnivorous zooplankton have been extensively studied by Anderson (1970). Combining all his data (1970) we have calculated a mean predation rate by omnivorous copepods of 7.2 prey/predator per day on prey < 1.0 mm during the first 24 h in a laboratory container. The extreme rate observed by Anderson was 25.2 prey/day per predacious copepod. Main (1962) while studying *E. lacustris* found that "the average daily number of brine shrimp (nauplii) consumed during a 1-mo period ranged from 3.2 to 5.6 for individually reared copepodites." Brine shrimp nauplii are considerably larger than the nauplii of *L. minutus*. For a predator to obtain the same amount of biomass by preying on the nauplii of *L. minutus* in Bluff Lake would require a much higher numerical predation rate. Confer (1971) found predation rates by *M. edax* on *A. floridanus* copepodites to be usually less than one per day. Predation on nauplii was not investigated, but from other studies cited previously one would expect the predation rate on nauplii to be higher.

Assuming size-selective predation and considering the observed rates of predation is it possible for the abundance of predacious copepods in Bluff Lake to account for the naupliar mortality of *L. minutus*? To account for all mortality for cohort 1 from N3 to N6 requires a predation rate of 14 prey/predator per day. To account for all mortality for cohort 2 and 3 from N1 to N6 requires a rate of 16 and 4.2 prey/predator per day, respectively.

The laboratory results encompass the rates necessary for predation by the copepods to account for all naupliar mortality of *L. minutus*. For the first two cohorts the necessary predation rates exceed the rate of most but not all laboratory observations. For the third cohort the predation rate by large omnivorous zooplankton necessary to account for all nauplii mortality is within the range most frequently observed in laboratory experiments. Initially we had no idea of the magnitude of predation accountable to the omnivores. The predation rates by the omnivores extrapolated from the rates observed most frequently in laboratory studies might have differed from the observed naupliar mortality by an immense magnitude. Instead, the normal predation rates observed in laboratory studies extrapolated to Bluff Lake (cohort 1 and 2) account for at worst about half of all naupliar mortality. Although we acknowledge the weakness of extrapolated calculations, we believe these results demonstrate that the omnivores caused a large portion of the herbivore mortality.

Dodson (1974) proposed that very high densities of large omnivorous zooplankton would lead to the complete elimination of small species of zooplankton. At moderate densities of large pred-

ators Dodson proposed that large and small species could coexist with the predators exerting a particularly significant effect on the early instars of the small species. The Bluff Lake assemblage of zooplankton is a mixture of large and small species, both of which may be influenced by the presence of a small population of brook trout (*Salvelinus fontinalis*). Perhaps moderate fish predation prevents the large omnivores from becoming sufficiently abundant to eliminate the small species of zooplankton. Of course, if the small species were absent only artificial manipulation could yield data to test Dodson's first hypothesis. In Bluff Lake the coexistence of a small species of herbivore with large species of omnivorous zooplankton has allowed us to estimate the in situ influence of size-selective predation on the smaller instars of the small herbivore. As with Dodson's data our calculations suggest that predation by omnivorous zooplankton on the early stages of a prey species could be the critical factor determining final abundance of a small herbivorous species.

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Effects of a Parasite, *Eubothrium salvelini* (Cestoda: Pseudophyllidea), on the Resistance of Juvenile Sockeye Salmon, *Oncorhynchus nerka*, to Zinc

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BOYCE, N. P., AND S. BEHRENS YAMADA. 1977. Effects of a parasite, *Eubothrium salvelini* (Cestoda: Pseudophyllidea), on the resistance of juvenile sockeye salmon, *Oncorhynchus nerka*, to zinc. J. Fish. Res. Board Can. 34: 706–709.

Most research to date on the tolerance of natural populations to physical and chemical stressors appears to have overlooked that parasites may themselves be stressors, and that they may be capable of modifying the resistance of the host to other applied stressors. Sockeye salmon (*Oncorhynchus nerka*) smolts trapped at the outlet of Babine Lake, central British Columbia, were subjected to 1 mg/ℓ dissolved zinc under laboratory conditions. Smolts infected with the intestinal cestode *Eubothrium salvelini* proved to be significantly more susceptible to zinc than were noninfected smolts. We recommend that future investigations of environmental effects, especially on wild populations, include consideration of the state of parasitism of the test organisms.

Key words: *Eubothrium salvelini*, cestode, sockeye salmon, Babine Lake, zinc, toxicity

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La plupart des chercheurs ayant étudié la tolérance des populations naturelles aux agents physiques et chimiques de stress semblent avoir oublié que les parasites peuvent être cause de stress et modifier la résistance de l'hôte à d'autres agents de stress. Des saumons rouges (*Oncorhynchus nerka*) à l'état de smolt attrapés à la sortie du lac Babine, dans le centre de la Colombie-Britannique, ont été placés dans un milieu contenant 1 mg/ℓ de zinc dissous, au laboratoire. Les hôtes du cestode intestinal *Eubothrium salvelini* se sont montrés beaucoup plus sensibles au zinc que les autres. Nous recommandons que les études des effets de l'environnement, spécialement sur les populations sauvages, tiennent compte du parasitisme.

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BABINE Lake in northern British Columbia is a major nursery area for sockeye salmon (*Oncorhynchus nerka*). Fry enter the lake shortly after emerging from the gravel in adjacent streams and migrate to sea as smolts approximately 11 mo later. Of the annual smolt output of this lake (approximately 30–70 million), about 30% are infected with an intestinal cestode *Eubothrium salvelini* (Schrank) (cf. Dombroski 1955; Smith 1973). The infection, acquired during the fry stage (Boyce 1974), is associated with reduced smolt size and vitality (Dombroski 1955; Smith and Margolis 1970; Smith 1973). Boyce (unpublished data) determined that *E. salvelini* infection impairs growth, vitality, and survival in juvenile sockeye salmon.

Babine Lake water contains certain heavy metals (Chau and Wong, unpublished data), of which zinc and copper are of particular interest as their ions are toxic to fish (Skidmore 1964; Sprague 1964) and because their levels are likely to increase as a result of intensive copper mining on the lake shore.

The high prevalence and demonstrated pathogenicity of *E. salvelini* in juvenile salmon of Babine Lake led to a preliminary examination of parasites as a possible factor influencing the resistance of fish to toxicants. This study compares the resistance of *E. salvelini*-infected smolts and their noninfected counterparts with zinc in solution.

Materials and methods — Sockeye salmon smolts trapped at the outlet of Babine Lake were sampled on site to establish the size category that would yield approximately 50% prevalence of *E. salvelini* infection. Consequently 600 smolts of 78–83 mm fork length were selected for air shipment to the Pacific Biological Station, Nanaimo. There they were held in dechlorinated fresh water with a total hardness of 13 mg/ℓ measured as $CaCO_3$ and a zinc concentration < 0.1 mg/ℓ. Water temperature was controlled at 10°C for the duration of the study. On the basis of a preliminary test, devised to establish a zinc concentration lethal to the experimental fish in 96 h, 1 mg/ℓ was selected as a suitable concentration for our experiment.

Fifty-five smolts were assigned to each of 10 30-ℓ fiberglass tanks with a water flow to each tank of 2 ℓ/min. A Technicon multichannel peristaltic metering pump (Technicon Inst. Corp., Tarrytown, N.Y. 10591) was adjusted to deliver a stock solution of zinc sulphate to the inlet pipes of five of the tanks at 1 ml/min, so that the final concentration in these tanks would be 1 mg/ℓ zinc.

The fish were provided a 3-day acclimation period in the tanks before the zinc was introduced. The required zinc concentration was reached in the treated tanks within 60 min of starting the metering pump. Fish were not fed during the acclimation and experimental periods. Mortality was noted at various intervals and the dead fish were weighed, measured, and examined for parasites within 24 h of death.

The 96-h experimental period was divided into 6-h intervals. The cumulative percent mortality at the midpoint of each interval was determined for *Eubothrium*-infected and *Eubothrium*-free fish. These data were plotted for each zinc-treated tank on log probit paper, regression lines were fitted by eye, and time to 50% mortality (LT50) interpolated from the graphs.

Results — During the 96-h experiment, mortalities in zinc-treated and control water were 96 and 8%, respectively.

The following parasites were recovered from 287 smolts exposed to zinc. Cestoda: *Eubothrium salvelini* (ovigerous adults), *Proteocephalus* sp. (plerocercoid stage), *Diphyllobothrium* sp. (encysted plerocercoids); Nematoda: *Philonema oncorhynchi* (subadults). *Eubothrium salvelini*, occurring in 55% of the fish examined, was by far the dominant parasite in terms of prevalence of infection and worm mass. In these respects the contribution of the other parasites was negligible and their occurrence showed no correlation, either individually or in combination, with the time to death of the smolts.

The mortalities occurring among fish in con-

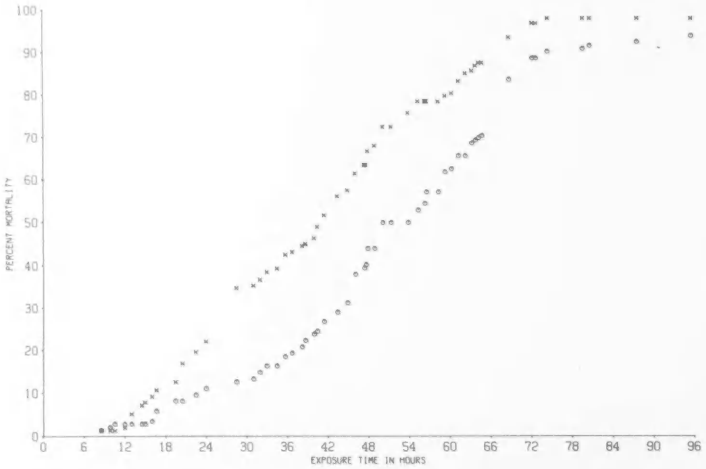

FIG. 1. Cumulative percent mortality of sockeye salmon (*Oncorhynchus nerka*) smolts exposed to 1 mg/ℓ zinc as a function of time. (Pooled individual resistance times from five tanks.) ×, smolts parasitized with *Eubothrium salvelini* ($N = 153$); ⓘ, *E. salvelini*-free smolts ($N = 134$).

trol tanks showed no correlation between parasitism and survival. On the other hand, *E. salvelini* infection was associated with earlier mortality in the zinc-treated tanks (Fig. 1). Time to 50% mortality (LT50) for *Eubothrium*-infected and *Eubothrium*-free sockeye salmon smolts exposed to 1 mg/ℓ zinc is shown below:

	Eubothrium	
Tank no.	Infected (*h*)	Free (*h*)
1	43	43
2	36	54
3	40	48
4	42	59
5	36	60
Mean	39.4	52.8
SD	3.28	7.25

Shortly before death the zinc-exposed smolts typically became dark and swam near the surface with erratic downward darting, occasionally displaying a cough reflex and loss of equilibrium. Evidence of hemorrhage near the anal fin and around the operculae was seen in postmortem examination of specimens.

Discussion — Considering the high survival of fish in the control tanks, mortality in the course of this study could not be ascribed to *E. salvelini* infection alone. On the other hand, zinc, at the dosage administered, proved lethal to virtually all exposed fish, with mortality hastened in the presence of *E. salvelini* infection. The appearance and behavior of the affected fish are in most aspects consistent with other observations of fish under chemical stress (Drummond et al. 1973; Adelman et al. 1976).

The mechanisms whereby zinc and *E. salvelini* independently stress salmon are not precisely known. The zinc ion is believed to cause damage to gill epithelia, resulting in asphyxia or osmotic stress (Lloyd 1960; Skidmore 1970); personal observations suggest the possibility that *E. salvelini* competes with the host for nutrients in the intestine, producing a condition tantamount to starvation. The manner in which these two agents together caused death of the test fish is not known; however, it is conceivable that a fish already weakened by a parasitic infection would more readily succumb to additional stress.

Despite numerous studies on the tolerance of natural populations to various environmental stressors, the effects of parasitism on the susceptibility of fishes to such stressors has been little investigated or even considered. Adelman et al. (1976) reported the expulsion of large numbers of cestodes by test fish as a reaction to the experimentally administered toxicant, but no concern was expressed for the possible effects of such an infection on the very tolerances being measured.

Horoszewicz (1972) reported that helminth infections reduced the resistance of bream to increases in water temperature, but her data provide

inadequate support for her thesis. Perevozchenko and Davydov (1974) found that yearling carp infected with the intestinal cestode *Bothriocephalus gowkongensis* were more susceptible to *p,p'*-DDT than were the noninfected.

We share the concern of the above authors that health factors, particularly parasitism, have been largely neglected in consideration of stress in wild populations. It is not the intent and design of this study to assign precise values to the susceptibility of our infected and noninfected fish to zinc; however, the results do demonstrate that significant differences can exist in this regard between infected and noninfected fish. We urge that future studies on environmental stress in wild populations include consideration of the state of parasitism of the subject animals.

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Fisheries and Aquatic Sciences[1] in Canada: An Overview

FISHERIES RESEARCH BOARD OF CANADA

Ottawa, Canada

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[1]Aquatic — water (marine, estuarial, and inland); sciences — mapping, monitoring, research, experimental management, application (development), and communication.

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Abstract

FISHERIES RESEARCH BOARD OF CANADA. 1977. Fisheries and aquatic sciences in Canada: an overview. J. Fish. Res. Board Can. 34: 710–727.

Scientific requirements and priorities are identified for the management of the fisheries and aquatic resources of Canada based on a series of background studies on scientific resources, scientific and technical information, renewable resource management (shelf seas, inshore seas, fresh water, fisheries rehabilitation, aquaculture), oceanography (physical, chemical, biological), aquatic environmental quality, and renewable resource utilization. Major priorities identified were: expanded fisheries rehabilitation programs on the Atlantic and Pacific coasts; oceanography of shelf seas, including the arctic; development of a national strategy for environmental monitoring; improved coordination of work on diseases, nutrition, and genetics related to fisheries rehabilitation and aquaculture; improved coordination and apportioning of federal and industrial research and development on the utilization of fisheries resources. The following aspects of fisheries and aquatic sciences need greater accent: development of national policy on fisheries and aquatic sciences; the provision of continuity for long-term research in federal laboratories; evaluation of federally supported research programs; collaborative arrangements for ecosystem and interdisciplinary research, including the social sciences; improved federal support for research conducted in universities; development of criteria for Canadian involvement in international activities; and a national information system related to diverse user needs. The national effort on fisheries and aquatic sciences was examined in terms of functional categories (mapping, monitoring, research, experimental management, application, communication) and institutional categories (international, federal, provincial, universities, industry). The Overview contains recommendations to improve the national capability in fisheries and aquatic sciences.

Résumé

FISHERIES RESEARCH BOARD OF CANADA. 1977. Fisheries and aquatic sciences in Canada: an overview. J. Fish. Res. Board Can. 34: 710–727.

Sont déterminés les besoins et les priorités scientifiques pour l'aménagement des ressources halieutiques et aquatiques du Canada, fondés sur une série d'études fondamentales des ressources scientifiques, de la documentation scientifique et technique, de l'aménagement des ressources renouvelables (mers épicontinentales et intérieures, eaux douces, revalorisation des pêcheries, aquiculture), de la qualité de l'environnement aquatique et de l'utilisation des ressources renouvelables. Les priorités ont été identifiées comme étant: l'élargissement des programmes de revalorisation des pêcheries des côtes de l'Atlantique et du Pacifique; l'élaboration d'une stratégie nationale pour la surveillance de l'environnement; une meilleure coordination des recherches sur les maladies, la nutrition et la génétique reliées à la revalorisation des pêcheries et à l'aquiculture; et une coordination et une répartition améliorées de la recherche et du développement par le gouvernement fédéral et l'industrie dans le domaine de l'utilisation des ressources halieutiques. On doit mettre davantage l'accent sur les aspects suivants des sciences halieutiques et aquatiques: l'élaboration d'une politique nationale à ce sujet; la continuité des recherches à long terme dans les laboratoires fédéraux; l'évaluation des programmes de recherche subventionnés par le gouvernement fédéral; les ententes de collaboration pour des recherches interdisciplinaires sur les écosystèmes, y compris les sciences sociales; l'appui accru du fédéral à la recherche dans les universités; l'établissement de critères concernant la participation canadienne aux activités internationales; et un système national d'information tenant compte des besoins diversifiés des utilisateurs. Les efforts réalisés à l'échelle nationale dans le domaine des sciences halieutiques et aquatiques ont été examinés en catégories fonctionnelles (cartographie, surveillance, recherche, aménagement expérimental, applications, communications) et institutionnelles (niveaux international fédéral, provincial, universitaire, industriel). Le survol du problème débouche sur des recommandations visant à améliorer les capacités du pays dans le domaine des sciences halieutiques et aquatiques.

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Recommendations

1. As a result of the extension of fisheries jurisdiction to 200 miles, the potential for rehabilitation of depressed fish stocks, the importance of fishing as a way of life, and the need to know the extent to which recovery processes are influenced by management changes, the Fisheries Research Board recommends that:

1.1 federal budget and manpower in fisheries and aquatic sciences be increased in selected activities to meet new and urgent responsibilities resulting from extension of jurisdiction to 200 miles;

1.2 an assessment or levy be made on all foreign vessels licensed to operate within Canada's 200-mile zone to defray, in part, the additional costs associated with extension of fisheries jurisdiction and Canada's responsibility for assuring that the research is adequate for management of its resources;

1.3 on the Atlantic coast, increased support be given to the development of a 5–20-year fisheries rehabilitation program, with a substantial proportion of the scientific effort, at least 50%, devoted to research needs;

1.4 as part of the Atlantic coast program, the Gulf of St. Lawrence Project be reviewed and implemented because of its high potential as a pilot project for improving our ability to manage major, marine, fish-producing ecosystems, and its major social implications;

1.5 on the Pacific coast, high priority be given to the development of a fisheries rehabilitation program for salmon, with an appreciable proportion of the total expenditure, at least 20%, devoted to research needs, including systems analysis and interactions between recreational and commercial fisheries.

2. As a result of the multiclient use of oceanographic knowledge, the increased accent on offshore and Arctic resources, the impact of weather and sea conditions on exploitation of those resources, the imminent extension to 200 miles of jurisdiction over resources, and the need to document the extent to which changes in fisheries are due to pollution or natural influences rather than exploitation, the Fisheries Research Board recommends that:

2.1 Canadian oceanographic activity in physical, chemical, and biological sciences be increased to an approximate doubling of manpower by 1986, with primary emphasis on research and monitoring of shelf waters;

2.2 a greater degree of coordination be exercised in the control over federal funding of oceanographic science, with provision for independent review.

3. Because of evidence of disruptions to the continuity and effectiveness of long-term research in federal laboratories involved in fisheries and aquatic sciences, and the lack of systematic procedures for evaluation of federally supported work, the Fisheries Research Board recommends that:

3.1 the Department of Fisheries and the Environment improve the scientific climate for research in its laboratories, with greater focus on resource-based issues, including both natural and social sciences;

3.2 provision be made for both internal and independent evaluation of long-term investigations in fisheries and aquatic sciences conducted with federal support.

4. In view of the essential role of universities in the national effort in fisheries and aquatic sciences, and the declining support in real terms for university-based research programs in these fields, the Fisheries Research Board recommends that:

4.1 the level of funding of subventions by the Fisheries and Marine Service, currently $360,000, and representing about 1% of in-house research expenditures, be increased to 5% of in-house research expenditures over a period of 3 years, with provision for support for periods up to 5 years to accommodate the needs of longer term investigations;

4.2 the subvention programs in fisheries and aquatic sciences of the Fisheries and Marine Service and the Inland Waters Directorate be brought under a single administration with provision for support of oceanographic investigations;

4.3 funds be made available for unsolicited proposals in fisheries and aquatic sciences from universities;

4.4 special support for research and training programs be given to a limited number of university centers of scientific strength in fisheries and aquatic sciences.

5. In view of the social and economic importance of Canadian water resources, the need for improved cost-effectiveness in monitoring aquatic environmental quality, the small effort devoted to biological monitoring, and the comparatively low proportion of effort devoted to the interpretation and use of data, the Fisheries Research Board recommends that:

5.1 a national strategy of environmental monitoring be developed and implemented;

5.2 federal/provincial agreements be secured with a view to apportioning and coordinating the research and monitoring effort related to aquatic environmental quality including the link with water flow;

5.3 there be a closer coordination between monitoring and research bases with a view to improving sampling design and the interpretation of data.

6. Because of the importance of knowledge of diseases, genetics related to breeding, and nutrition to both fisheries rehabilitation and aquaculture, the Fisheries Research Board recommends that:

6.1 a national program be established on diseases, genetics, and nutrition, accenting wherever possible cooperation and joint use of facilities with research workers in governments and universities.

7. Because of the inadequate interpretation and transfer of scientific and technical information on fisheries and aquatic sciences in terms of diversified user interests, and the fragmented approach to the acquisition, interpretation, and dissemination of information, the Fisheries Research Board recommends that:

7.1 a Canadian aquatic sciences and fisheries information system be established in the Department of Fisheries and the Environment for the access, interpretation, and processing of information and data relating to aquatic sciences and fisheries in Canada, interfacing with relevant national and international systems;

7.2 this system have an analysis and interpretation function to organize and prepare information in forms suitable for various users;

7.3 a regional capability be developed as part of the activities of this system;

7.4 an applied periodical be published that interprets developments in fisheries and aquatic sciences and presents these in a form understandable by industry, managers, and other users.

8. Because of the increasing importance of communicating knowledge of fisheries and aquatic sciences to fishermen, nonscientific professionals, and the general public, the Fisheries Research Board recommends that:

8.1 a publication similar to *Fisheries of Canada* be reestablished;

8.2 the Department of Fisheries and the Environment more actively interest and inform the public on scientific and political aspects of complex issues pertaining to fisheries and aquatic environments;

8.3 the Department of Fisheries and the Environment prepare understandable accounts of existing federal and provincial legislation and the responsibilities of key agencies in the area of aquatic environmental quality.

9. Recognizing that the effectiveness to industry of the Fisheries and Marine Service Resource Utilization Program has been called into question, the need for this program to become more responsive to industrial requirements, and the importance of technical research assistance to the primary industry and small companies, the Fisheries Research Board recommends that:

9.1 the Resource Utilization Program of the Fisheries and Marine Service accent research and development of fisheries technology related to harvesting, processing, storage, quality control, underutilized resources, and food and nutrient values;

9.2 a regional advisory committee be formed for each center associated with the Resource Utilization Program with advisory control over industry-related investigations;

9.3 the effectiveness of the Resource Utilization Program be reevaluated in 1980 on the basis of receptivity and financial support by its clients, in particular the fishing industry.

10. In view of the need to maintain a reasonable level of aquacultural activity in order to take advantage of new developments as they arise, and limitations to aquaculture imposed by Canadian climate, the Fisheries Research Board recommends that:

10.1 Fisheries and Marine Service activities in aquaculture be maintained at current levels for the present;

10.2 research and development programs in aquaculture be nationally coordinated and accent technology transfer.

Introduction

In 1973 the Fisheries Research Board of Canada assumed an advisory role to the Minister of Fisheries and initiated a review of scientific requirements and priorities for management of aquatic environments and fisheries. The perspectives were national and international, natural sciences and social sciences, commercial and recreational, short-term and long-term. The emphasis was on research.

The Board organized for this task by establishing standing committees on Priorities, Scientific Resources, and Scientific Publications and Information, and study committees on Resource Management, Oceanography, Environmental Quality, and Resource Utilization. In some cases, external experts contributed to the work of the committees. The composition of the committees is set forth in Appendix I.

The Committee work was based on a com-

missioned series of background studies on Scientific Resources (Present Situation and Needs), Scientific and Technical Information, Resource Management (Shelf Seas, Inshore Seas, Fresh Water, Rehabilitation, and Aquaculture), Oceanography (Physical, Chemical, and Biological), Environmental Quality (Problems and Toxicology), and Resource Utilization. The background studies, following reviews by the appropriate Committee and by the Board, have had limited distribution in manuscript form. Most of them have been published as Perspectives in the *Journal of the Fisheries Research Board of Canada* and in the bilingual *FRB Report* series. The titles and authors are identified in Appendix II.

Board interim reports on each subject were presented to the Minister and senior officials of Environment Canada and are set forth in Appendix III. Progress reports and interim recommendations have appeared in the *Annual Report* of the Board for the years 1973–76.

The content of the Overview has been developed and approved by the Fisheries Research Board. Its preparation has been carried out under the supervision of the Board's Priorities Committee. The text of the Overview was written by Dr W. R. Martin, Senior Scientific Advisor to the Board Chairman. The individual Board reports, which appear as Overview Appendices, were prepared by the committee chairmen identified in Appendix I.

The Overview is broad in scope, but not exhaustive in coverage. A number of important topics such as the arctic, international science, recreation and sportfishing, social science, research climate, capital facilities, and energy costs have received little or no attention. Based on a 10-year perspective, the Overview advocates an orderly expansion of fisheries and aquatic sciences in Canada to meet increased national responsibilities and proposes opportunities for an improved focus and balance on national scientific resources and programs.

The Challenge

The principal challenge facing Canadian fisheries and aquatic scientists is *stewardship* of extensive aquatic resources for the benefit of Canadians.

Canada has a greater area of fresh water, a longer coastline, and a larger continental shelf area than most countries of the world. Canada is bordered by three oceans and the St. Lawrence waterway, which stretches halfway across the continent. These geographic features provide unrivaled potential for aquatic food production, recreation, supplies of potable water, low-cost water transportation, energy production, and socioeconomic opportunities for coastal communities.

These aquatic resources exist in a cold climate, with the associated complications of winter and ice cover. They are also subject to the ubiquitous problems of growth of human uses of water. There are increasing conflicts in sharing water for commercial fisheries, recreation, domestic consumption, energy production, industrial cooling, waste disposal, and transportation. There are growing pressures for short-term gains by a few at the expense of long-term benefits for all. The greatest concerns are the impacts of pollution and overexploitation of aquatic resources.

Canadians have special responsibilities to achieve effective conservation and well-balanced multiple use of aquatic resources in this era of accelerating change and uncertainty, in which confrontations and crises can be expected to appear with increasing frequency. The national and international responsibility for stewardship implies accountability and credibility to Canadians, to the world, and to future generations. This requires high levels of scientific effort and competence to provide the knowledge necessary for conservation and rational utilization.

Demands on aquatic scientists have been accelerating. Responsibilities have broadened to include the arctic, Exclusive Fishing Zones, and now Economic Zones. Cooperative international shelf-seas science is being replaced, in large measure, by coastal state responsibilities. Fisheries objectives have evolved from emphasis on development to a new emphasis on rehabilitation, with greater requirements for resource assessments, forecasts, and resource allocations. There is growing interest in the culture of living resources in fresh water and salt water. With deterioration of aquatic environmental quality, it has been necessary to adjust priorities and to redirect some scientific effort to antipollution research. In general, as Canadian aquatic interests have expanded, scientific questions have broadened from fisheries, defence, and energy, to include recreation, transportation, underwater exploitation of nonrenewable resources, environmental quality, and weather forecasting. In all these areas, scientists are asked to provide the knowledge base, predictive capability, and management expertise required to protect and make better use of Canadian waters.

Since financial support of fisheries and aquatic sciences is not sufficient to fully meet all these demands, it is necessary to identify priorities, and to find ways of making better use of available scientific resources.

Mandates

To preface the identification of priorities, it is useful to summarize relevant objectives, policies, and commitments.

Within the national *objectives* of environmental harmony, material well-being, and cultural opportunity, fisheries and aquatic resources can provide Canadians with opportunities for livelihood and personal fulfillment. This is particularly important in regions where Canadians have few alternatives to dependence on fisheries and water for their livelihood. Although economic development and employment receive primary consideration in fisheries and aquatic sciences, the human demands for water, proteins for food, recreational facilities, and fishing as a way of life are also of major concern.

In addition to federal Acts pertaining to fisheries and aquatic sciences, Canadian "Oceans" and "Commercial Fisheries" *policies* provide priority guidelines. The key directive of the Oceans Policy is to develop and control, within Canadian borders, the essential elements for exploiting offshore resources through the development of science, technology, and industry. The aim is to achieve excellence in operating on and below ice-covered waters and to develop an information base on renewable and nonrenewable offshore resources equal or superior to those of foreign governments or large multinational corporations. The Commercial Fisheries Policy states that the guiding principle in fisheries management has changed from maximization of biological yield to best use of society's resources, including labor, capital, and fish stocks. The government intends to create a strong and stable fishing industry, providing secure and rewarding livelihoods to all participants.

Current national *commitments* that have an impact on fisheries and aquatic science priorities are: (1) the extension of jurisdiction over fisheries from 12 to 200 miles beyond baselines, effective January 1, 1977; (2) a Salmonid Enhancement Program for the Pacific coast, involving rehabilitation of salmon resources to former levels of abundance; (3) commitments to more than 12 international commissions and numerous multilateral, bilateral, and regional agreements; and (4) anticipated increasing responsibilities for pollution control and marine scientific research, resulting from the Law of the Sea Conference.

These mandates call for a scientific knowledge and predictive capability to manage the quality, productivity, and multiple use of the aquatic environment, with emphasis on *rehabilitation*, *allocation*, and *utilization*.

On the east coast, the highest priority for marine work arises from the extension of jurisdiction to 200 miles. In fresh water, the highest priority is rehabilitation and protection of the environment and renewable resources of the huge St. Lawrence waterway. On the west coast, the highest priority is rehabilitation of Pacific salmon.

Program Areas

The board has examined science priorities in renewable resource management, oceanography, environmental quality, and resource utilization. These areas are discussed briefly as they relate to the Overview perspective.

Renewable Resource Management

The major tasks facing managers of marine, anadromous, and freshwater fisheries are rehabilitation, allocation, and rational utilization of resources.

The major challenge is to meet requirements inherent in responsibility for a 200-mile fisheries zone. On the Atlantic coast, this involves the addition of more than half a million square miles of water. Management requirements include regulations, surevillance, enforcement, public information, and above all, fisheries science.

It is urgent to develop, over the next 10 years, a scientific program for rehabilitation and allocation of the fisheries of the east coast. The first requirement is to recognize and institutionalize the scientific base for resource management: mapping, monitoring, research, and experimental management. The program should include social and natural sciences, and their synthesis into models to assist the management of fisheries. Joint participation with provincial agencies, universities, and industry should be planned from the outset to make best use of national expertise. An expansion of shelf-seas fisheries science should be achieved over a 5-year period.

In the northwest Atlantic, Canada has depended, in part, on fisheries science conducted by other nations. This scientific effort can be expected to fall off proportionately to decreased foreign fishing, leaving Canadian effort grossly inadequate for credible management. Provision of additional funds for relevant fisheries science will be necessary; scientists should be transferred and retrained for high-seas assignments; collaboration with universities and provincial agencies should be sought; and chartered vessels and ships of opportunity should be engaged until obsolete research vessels can be replaced with modern craft and unmanned monitoring instrumentation.

On the Pacific coast, the major fisheries challenge is salmon enhancement and management. Doubling the production of the resource through rehabilitation appears possible. The main thrusts should be given to small localized operations and simple devices for habitat improvement. The management of Pacific salmon requires large and continuing inputs of science. An experimental management approach is called for, with careful planning and assessment of each step in the development of the enhancement operation. At least 20% of the proposed expenditures should be assigned to research, including such topics as: classification of lake and stream types and assessment of their productive capacities; methods of culture; experiments with new techniques; nutrition, disease, and genetics; competition with natural stocks; and ocean pasturing. Collaboration among disciplines, functions, and institutions should be fostered as a means of ensuring program success.

In the arctic, the dominant renewable resource management interest is marine mammals. With growth and spread of human activity in the north, seals and small whales are increasingly vulnerable to overexploitation and pollution. To date, ecological and social science studies have been small scale and spotty. Increased research on the production, distribution, mortalities, and utilization of aquatic living resources is crucial to current and anticipated requirements for management of arctic fisheries.

Inland freshwater fisheries have suffered through environmental degradation and overexploitation. To restore and expand these fisheries, managers require strong input from fisheries and aquatic scientists, particularly with respect to: (1) ecosystem investigations; (2) interdisciplinary studies; (3) collaboration among federal, provincial, and university scientists; (4) concentration of research on selected river systems or lakes; and (5) an experimental approach to management.

Within the southern regions of British Columbia, Manitoba, Ontario, Quebec, and New Brunswick there are a number of institutions that could profitably collaborate in freshwater fisheries science. Attempts should be made to establish collaborative arrangements centered on Vancouver, Winnipeg, Toronto, Quebec, and St. Andrews. Communication, nationally and internationally, with marine and fisheries scientists will accelerate freshwater rehabilitation programs.

Aquaculture, although not of high priority at the moment, will probably be so in the future. With growing interest in sportfishing and gourmet cooking, a slowly increasing proportion of aquatic renewable resources will be produced by farming, rather than ranching and hunting. In recognition of the long lead time required for development of the relevant science and technology, present levels of aquacultural activity should be maintained. Careful policy development, and planning of facilities and programs will be needed to avoid the fragmentation and duplication commonly experienced in renewable resource programs.

Long-term scientific requirements for rehabilitation of freshwater and anadromous fisheries and for aquaculture lie in the fields of diseases, nutrition, and genetics related to breeding. To minimize the high costs of this research and ensure its orderly development, a national program is needed to avoid unnecessary duplication, and to make best use of federal, provincial, and university expertise, including agricultural and veterinary scientists.

Oceanography

Ocean science, like atmospheric science, serves a variety of interests. In the federal government alone, agencies requiring oceanographic information include Fisheries, Atmospheric Environment, Transport, Energy Mines and Resources, Regional Economic Expansion, National Defence, and Public Works. Although post World War II expansion of oceanography has been effective and of high quality, it has not been possible to satisfy all the demands.

The Board has examined physical, chemical, and biological oceanography in relation to the extension of jurisdiction to 200 miles, energy production in the arctic and on the continental shelf, and the extent to which changes in fisheries are due to pollution and natural oceanographic influences. The main conclusions are: (1) oceanographic sciences should be increased by an approximate doubling of manpower by 1986, with primary emphasis on shelf seas, including the arctic; (2) applied oceanographic services should be established by the early 1980s, to provide large-scale information and predictions of oceanographic conditions; (3) Canada should keep its options open for a future share of the high-seas resources of the world by participating in international research projects; (4) emphasis should be given to oceanographic information bearing on fish production and distribution; and (5) a greater degree of central control is needed over federal funding of oceanographic science, with provision for coordination by a strong committee of users and doers, and reviews by an independent agency.

On the Atlantic coast, oceanographic facilities

have expanded in an effective way. The area of responsibility from the Bay of Fundy to the eastern arctic is enormous and current funding for oceanographic services and vessel operations is inadequate. Quebec is developing competence in oceanography, and should be encouraged, possibly through contracts, to further collaborate in the oceanographic work required in the St. Lawrence estuary and Gulf.

The Pacific and western Arctic oceans are of great economic and climatic importance to Canada and a large increase of west coast oceanographic knowledge is needed. Improved oceanic and atmospheric weather forecasting is an important long-term objective. The relevance of arctic oceanography to exploitation of offshore mineral resources is becoming increasingly apparent.

Environmental Quality

Preservation of the quality of the aquatic environment is of continuing high priority, since our capacity to pollute waters increases with growth of human population and changing technology. The key requirements are rehabilitation of polluted waters, environmental impact studies, and control of existing and potential contaminants.

The Board examined the national scientific effort on aquatic environmental quality, its present state, current trends, future implications, monitoring programs, and research. This examination revealed an almost complete lack of a philosophy and strategy for environmental monitoring. Evidence was found of duplication in federal and provincial monitoring programs, too much chemical monitoring and too little biological monitoring, insufficient attention to nonpoint sources of contamination and to the arctic, unnecessary levels of secrecy that encourage duplication and inhibit coordination, inadequate interpretation of data, and ineffective coordination of the monitoring of water quality and flow.

The priorities are to: (1) develop a national strategy for the monitoring of aquatic environmental quality; (2) secure federal/provincial agreements with a view to apportioning and coordinating the research and monitoring effort; (3) improve the links between research and monitoring to improve sampling design and interpretation of data; and (4) increase the biological component of the monitoring program. In addition, a publication, periodically updated, is needed that describes in simple language the federal, provincial, and municipal agency roles and legislation pertaining to environmental quality for the use of industries, governments, and citizens. Even experts find it almost impossible to disentangle the mix.

Renewable Resource Utilization

Canada has had an effective scientific program of resource utilization. The benefits have accrued to fishermen in the form of greater financial returns for their catches, to the secondary fishing industry through lower costs and higher market value for their products, and to consumers in terms of the safety, quality, variety, and cost of aquatic foods and natural products.

A survey of the clients of the federal resource utilization research and development program revealed considerable apprehension concerning the: (1) relevance of federal investigations to the secondary industry; (2) cost/benefit ratio of technological science; (3) priority in relation to other scientific needs; (4) low level of contracting out to provincial research organizations, industry, and universities; (5) inadequate transfer of scientific information to industry; (6) low levels of research and development in the fishing industry; and (7) almost complete dependence on the federal government for the conduct and financing of investigations.

Because of the common property nature and variety of fisheries resources, the fragmentation of the fishing industry, and the interests of consumers, it was concluded that some federal responsibility for technological research and development (R&D) is unavoidable. Nevertheless, since renewable resources become private property once caught, the fishing industry should assume a fair share of responsibility for the relevant science. This should be achieved by greater industry participation in R&D, and through appropriate financial support of government R&D by industry.

The Board concluded that the federal resource utilization research program should continue, for the present, at the current level of expenditure, using existing technological facilities, contracts, and greater collaboration with industry, including greater use of their facilities. To ensure that the program becomes more responsive to industrial requirements, federal laboratories should accent projects related to harvesting, processing, storage, quality control, underutilized resources, and food and nutrient values. Regional advisory committees should be formed for each center, with advisory control over industry-related investigations.

The effectiveness of the resource utilization program should be reevaluated in 1980 to assess

its usefulness to clients, and to consider an appropriate division of financial support by those benefitting from these investigations.

Functions

Fisheries and aquatic sciences have been examined in terms of the functional categories of mapping, monitoring, research, experimental management, application, and communication.

Mapping

Mapping is well-developed and organized in the Canadian Hydrographic Service of the Fisheries and Marine Service, in comprehensive regional map libraries, and in the Atlas of Canada. Biological, fisheries, and environmental quality maps should be included with the physical maps. Relevant expertise should be co-opted to bring together existing aquatic biology and fisheries maps, update them, develop a balanced series of new maps, and integrate them into regional map libraries.

Monitoring

Monitoring of temporal changes in fresh and marine waters provides the basic data needed for interpretation, prediction, and manipulation of natural and man-made changes in the environment and renewable resources. This is an essential and expensive operation.

Sonic surveys, sampling of year-classes before they enter the fisheries, catch/effort data, quantitative statistics of catches and landings by sizes and ages, and socioeconomic information are basic tools for application of science to management and harvest protocols. Total allowable catches, license limitations, and other measures to achieve best use of labor, capital, and resources are based on analyses of such information by natural and social scientists. A substantial increase in effort will be needed to take over the monitoring responsibility previously coordinated and compiled by international fisheries commissions.

The large national effort on monitoring of water quality is plagued with problems of jurisdiction, duplication, secrecy, redundancy, oversampling, precision and standardization of methods, and lack of interpretation. Aquatic environmental monitoring should be systematized, with greater use of biological monitoring. More responsibility should be assumed by provincial governments, and some of the work should be contracted out to provincial research organizations and to industry.

Research

Research provides the knowledge for understanding, predicting, and managing the aquatic environment and its resources, and the way we use them.

Internationally, Canada has had a good reputation for the quality and quantity of its fisheries and aquatic research. Nationally, however, apprehension is growing about the high costs and the relevance of this research to the needs of the country. To be realistic, much more attention must be given to ways of increasing research effectiveness at reasonable costs.

The Board considers that much of the research effort should be devoted to *short-term* (immediate to 5 years) problems of fisheries and aquatic management. Through assessments, synthesis, and modeling, research centers should seek to understand, simulate, and explain major natural and man-made events and processes. The models should encompass social, economic, and ecological components, and should facilitate prediction of the consequences of various management options.

Equally important is the research effort devoted to *long-term* investigations (5–20 years). Examples of problems requiring long-term investigations are the biological productivity of representative ecosystems, the best stage in the food chain at which the resource should be harvested, and the transport of contaminants through air, water, and aquatic food chains. Emphasis will have to shift from research by disciplines and by species to interdisciplinary research on ecosystems. This is needed to understand how ecosystems work and to provide bases for their manipulation and best use by man. Such research should be concentrated on selected bodies of water, where scientific principles can be developed for general application.

The Board considers that an interdisciplinary study of the Gulf of St. Lawrence has a high potential value as a pilot project to test our capability to rejuvenate and manage a productive marine ecosystem of major economic and social importance. The results are relevant to the new responsibility of managing the east coast fisheries and marine environment. Special attention should be given to ocean dynamics and interfaces, assimilative and productive capacities, natural and pollution factors affecting aquatic ecosystems, natural and fishing impacts on renewable resource recruitments and mortalities, and the socioeconomic dimensions of the fisheries.

Management and research "climate" are crucial to effectiveness of research. The separate roles, functions, capacities, and long-time frame for

research require a separate organizational identity if it is to thrive. Under pressure of crises and short-term priorities, it is easy to stop or postpone long-term research, without fully appreciating the slowness of recovery and the long-term impacts of such action. Common administrative services, such as purchasing and contracting, and personnel, which have evolved to enhance overall government efficiency, introduce serious constraints to the effectiveness of research managers. The use of intermediaries between scientists is like making love through a broker.

One of the constraints to fisheries and aquatic research is fragmentation of national effort inherent in a large number of federal, provincial, and university centers. Although research programs are generally good, they are small scale and not well coordinated, with consequent duplication of effort in some areas. To correct this situation, most research should be concentrated in a few interdisciplinary centers of scientific strength, which are oriented to regional problems and draw on expertise from governments and universities in collaborative research projects. For full effectiveness research centers should be linked nationally.

Experimental Management

Experimental management should receive greater attention than heretofore. The abundance of lakes, rivers, and undeveloped marine coastlines is such that a large number of sites can be designated for management experiments. There are also many opportunities in management programs to include experimental projects as part of a learning process and management development. Most fisheries and water management projects would move ahead more rapidly and effectively if treated as hypothesis testing experiments, with appropriate attention to design, implimentation, assessment, evaluation, and documentation of results.

A number of sites have been used effectively for management experiments, e.g. experimental lakes in Northern Ontario for eutrophication and impact from air pollutants; lakes in New Brunswick and British Columbia for fertilization to increase fish production; Carnation Creek, British Columbia, for effects of logging; St. Mary's River, Newfoundland, for pink salmon transplants; Fatty Basin, British Columbia, for lobster transplants; and prairie ponds for rainbow trout farming. Expanding this productive approach will be increasingly difficult, unless there is early allocation of special areas for management experiments on resource use conflicts, environmental impacts, resource rehabilitation, and aquaculture.

A variety of resource management experiments has been carried out with varying degrees of scientific control, e.g. rotational closures of small trout lakes, large meshes in trawl nets, wide lath spacing in lobster traps, hatcheries and spawning channels, fish used as a direct source of human protein rather than fish meal (e.g. herring and capelin), and experimental "overfishing" of a prescribed yellowfin tuna fishing area. It will be increasingly useful to carry over such experimental management approaches to major problem areas, such as resource rehabilitation and allocation, fishing effort control, harvesting and processing, and control of environmental quality. It must be realized that the only way to answer some of the burning questions of management is to try out some new kinds of management and evaluate them as to causes and effects.

Experimental management should be vigorously pursued by capitalizing on opportunities, and by fostering collaborative involvement amongst scientists, managers, and industry. Fisheries biology must muture into a virile fisheries science.

Application (Development)

Application of fisheries and aquatic sciences embraces a wide spectrum of science services, technology transfer, and development activities essential for effective management, and basic for public and industry support.

Prediction services which are now provided on a limited scale for ice cover and marine weather should improve and expand during the next decade to meet the demand for forecasts of water "climate" and quality, and of resource availability and quality.

Resource development, which includes resource rehabilitation, maintenance, enhancement, and culture, is a major activity of fisheries and aquatic sciences. Canadian developments of fishways, hatcheries, spawning channels, transplants, and aquaculture, which have received international recognition, have resulted from the quality of the fisheries research backing up this applied activity. A large potential for application of existing and new knowledge to resource rehabilitation still remains.

Industrial development has been a major activity in Canada. A major part of the industrial development work has involved testing, adapting, and demonstrating resource harvesting methods and equipment developed elsewhere in the world. Some of the technology transfers have been car-

ried out scientificially with pilot-scale experiments, preproduction runs in commercial facilities, and adequate reporting of results. This has been particularly true for the technological work on smokers, dryers, fish holds, refrigerated sea water, processing lines, and frozen storage. Canadian fishing efficiency is now relatively high, and the continuation of this status will depend to an increasing degree on the scientific content of the industrial development program.

Communication

The exchange of information between scientists and managers, industry, general public, and other countries is a weak link in the chain of science functions.

Although the need to make scientific knowledge available to users has been increasing, widely understandable scientific writing has been decreasing. This has been caused through deference to other pressures on scientists and by inadequate funding for applied publications, such as *Fisheries of Canada* and the *Fisheries Research Board of Canada Bulletin* series. One bright spot has been the production of review papers, as Perspectives in the *Journal of the Fisheries Research Board of Canada* and the separate distribution of some of these as *FRB Reports*.

A Canadian periodical on the interpretation and application of fisheries and aquatic sciences is badly needed. The periodical should be broad in scope, bilingual, appealing, and easily understandable to nonscientific audiences that could make practical use of it. Increased attention to other forms of interpretive writing such as bulletins and circulars is also needed to improve the balance of scientific and applied publications. Applied publications are so important that action should not await budgetary increases.

An important step in the development of communication would be to form a *Canadian aquatic sciences and fisheries information system* (Casfis). The system should be tied into the National Scientific and Technical Information (STI) network and the newly formed international Aquatic Sciences and Fisheries Information System (ASFIS). The system would provide a national focal point for scientific and applied publications, an online data base for storage and retrieval of information, and an analysis and interpretation function. Information, acquisition, screening, and referral services should be included in this information system.

To effectively transfer fisheries and aquatic sciences information to users, Canada should have an appropriate *extension* activity. The successes of agriculture extension services in Canada and the United States provide a basis for development and support of a similar service for fisheries and the aquatic environment. Professional staff with interpretative abilities and science writers are needed to provide the link between scientists and their clients. A variety of extension activities, such as short courses, government/industry seminars, and interpretation centers should be reconsidered in the development of an extension function.

Institutions

Responsibilities for fisheries and aquatic sciences have been considered in terms of international, federal, provincial, university, and industry categories, and their interfaces.

International

Canada has had a high competence and reputation in fisheries and aquatic sciences. This valuable asset should not be allowed to slip. Canada can continue to benefit greatly from the international credibility of its aquatic sciences, from the global intelligence that is brought to bear on Canadian issues, and from the collaboration of international experts in dealing with Canadian scientific problems.

International communication, cooperation, and collaboration are crucial to fisheries and aquatic sciences. The geographic dimensions are global, the human and aquatic ecosystem problems are largely universal, and the science is international. The demands are too great and the scientific resources too small to do it alone.

Growing disparity between developed and developing nations is of increasing concern to international understanding and world order. Commitments to foreign aid will be met, in large measure, by providing scientific and technical assistance to developing nations, thereby enabling them to help themselves.

However, the effectiveness of Canadian involvement in international fisheries and aquatic sciences at both nationally controlled and internationally controlled levels should be evaluated. Criteria for Canadian involvement in international scientific arrangements should be developed.

Federal

The federal government is responsible for Canadian ocean science, including mapping, monitoring, research, and the professional leadership involved in experimental management, application, and communication. Wherever possible,

contracts, subventions, facility sharing, and collaborative programs should be used to make use of the best available expertise in governments, universities, institutes, and industry. This should apply also to those components of freshwater science that are federal rather than provincial responsibilities.

The national responsibility for fisheries rests with the federal government, and this responsibility has greatly increased by extension of fisheries jurisdiction to 200 miles from coastal baselines.

To coordinate research and to avoid duplication and even conflicts, Canada is obliged to assume responsibility and leadership for the international research effort within the 200-mile zone. Some of the research effort by foreign nations fishing in Canadian waters may be sufficiently relevant to be considered part of their contribution to the costs of scientific management. Canada should support a major part of the scientific costs since the long-term benefits will accrue to Canada.

With extension of jurisdiction, consideration can be given to the nature of ownership of marine resources. It should now be possible to develop private property concepts and a user-pays principle, using licensing revenues to defray some of the costs of fisheries and aquatic research. Major resource rehabilitation programs, such as those for Atlantic groundfish and Pacific salmon, should include cost recovery objectives, with a reasonable percentage of revenues for relevant research.

A separate identity for research in fisheries and aquatic sciences is needed to ensure that long-term problems are addressed. Research requires special talents and styles, evaluation mechanisms, and highly trained personnel who need freedom for resourcefulness and creativity. Separate program and cost controls for research should be maintained at both national and regional levels. In this accounting, the expanding functions of science service, experimental management, development, and communication should be clearly identified in budgets, and distinguished from scientific research. Research activities and budgets can be most effectively managed by giving research an identity, to assure the continuity and insulation, without isolation, required for a well-balanced and effective program.

Internal evaluations of federally sponsored research programs should be carried out regionally and nationally each year. External evaluations should be conducted periodically under the supervision of an independent agency such as the Fisheries Research Board. Under such arrangements, each laboratory program would be subjected to an internal review annually and an external review about every 5 years.

Provincial

Responsibility for freshwater fisheries resources has been delegated by the federal government to most provinces. Coastal provinces share responsibility with the federal government for diadromous and tidal fisheries resources.

For waters that straddle international and interprovincial boundaries, fisheries resource responsibilities are shared by the appropriate governments. In general, the provinces assume a greater share of the national responsibility for fisheries at the development end of the science spectrum. Provinces have an even greater share of the national responsibility for aquatic environmental quality. In varying degrees they carry out the whole range of monitoring, research, experimental management, application, and communication functions of fisheries and aquatic sciences.

Provinces should be encouraged to assume a greater share of the national responsibility for fisheries development and environmental quality. By increasing their technical strengths, provinces could collaborate with other governments in carrying out major programs to meet national objectives. Systematization of environmental monitoring deserves special attention.

Universities

In the first part of this century, universities carried out most of the fisheries and aquatic research in Canada. University centers of scientific strength in fisheries research have declined in recent years for lack of government funding. During the past two decades, university contributions to oceanography and limnology increased, aided by negotiated development grants in support of a few centers of scientific strength.

The successful development of oceanography in Canadian universities provides useful guidance for development of a broader university role in fisheries and aquatic sciences. The primary considerations are: (1) identification and development of a small number of university centers of scientific strength, preferably in close proximity to related government centers; (2) interprovincial and interuniversity participation in such identification and allocation; and (3) federal support of university staff and students, in selected universities, through seasonal student employment, development and operating grants, research agreements or subventions, contracts, collaborative research projects, sharing of expensive computer, water laboratory and research vessel facilities, and establishment of chairs for which outstanding leaders can compete.

The demand for fisheries and aquatic scientists will increase, in response to increased responsibilities for the 200-mile zone and foreign aid. There will be a continuing need for research scientists, and an even greater need for other scientists to carry out the monitoring, prediction services, research support, experimental management, development work, and interpretive science communication. Accordingly, increased attention should be given by universities to the interdisciplinary training of fisheries and aquatic scientists.

Universities can also make major contributions to research in such areas as aquatic ecosystems, environmental quality, fish health, social sciences, and systems analysis and modeling.

Although there is a growing awareness of the need for broader environmental studies, interdisciplinary research is constrained by the traditional separation of natural, social, and health sciences in universities and governments. Strong support of a real experiment in interdisciplinary research might facilitate a break-away from tradition to more effectively meet changing needs in fisheries and aquatic sciences.

The level of subvention funding of fisheries and aquatic sciences in universities should at the very least be doubled immediately, with provision for further growth to about 5% of in-house research expenditures, and support on 3–5 year bases, to foster longer-term investigations. The expanded program should include support of oceanographic research.

Industry

Industries involved in fisheries and water quality investigations have been dependent on government and provincial research organizations for scientific and technological support. It is argued that this is so because of the common property nature of the resource base, and because of the fragmentation of industry. But this situation is changing, as resources are brought under national control and allocation, and as industry increases its capacity for collective action.

Industry can and should collaborate in experimental management, and development science. This can be accomplished either by financial support, or preferably by active participation, including provision of commercial vessels and shore-based facilities to scientists. A substantial increase in joint government/university/industry projects is needed. With rehabilitation of resources, controlled entry, and resource allocation, foreign and Canadian industry should be in a position to pay appropriate license fees to finance part of the cost of aquatic resource science and management.

Industry will need a greater technical competence if it is to achieve the economic efficiency that comes with application of technology by professionals, and to maximize the benefits of introducing science into industrial practice. This might be achieved, in part, through industry support of provincial research organizations. To foster this objective, a larger percentage of the federal budget for fisheries and aquatic science should be contracted out to industry and provincial research organizations.

Conclusions

Priorities

1. There is a need for a statement of national policy on fisheries and aquatic sciences. This is important to permit the establishment of priorities and strategies; the apportioning of effort among governments, universities, and industries; and a rationale for the proportion of effort devoted to short-term and long-term investigations.

2. High priority must be given to the fisheries science required to meet Canada's increased responsibilities for management of the 200-mile zone. The objectives of rehabilitation and rational utilization of the resources necessitate that the federal effort in terms of manpower for fisheries science on the continental shelf be increased by about 50% by 1981, with the additional costs defrayed, in part, through license fees, particularly for foreign vessels operating within Canada's jurisdiction.

3. On the Atlantic coast, increased support is needed for the development over 5–20 years of a shelf-seas fisheries rehabilitation program to rebuild stocks, with at least half the scientific effort devoted to research. This program should include provision for improved research vessel capability, and an interdisciplinary study of the Gulf of St. Lawrence as a pilot project with implications to managing the east coast fisheries and marine environment.

4. On the Pacific coast, high priority should be given to the projected 300 million dollar federal program for rehabilitation of salmon resources. This will require large and continuing inputs of science with at least 20% of the total expenditure devoted to research.

5. High priority should be given to oceanography of shelf seas, including the arctic, because of its relevance to the development of energy and fisheries resources, and to the effects of pollution on the coastal waters. There is need for a 50% increase in oceanographic activity by 1981, making more effective use of research vessels and unmanned monitoring instrumentation.

6. In the area of aquatic environmental quality there is a need for a national philosophy and strategy on environmental monitoring with clearer apportioning of federal/provincial responsibilities to avoid duplication, closer ties between research and monitoring, and greater emphasis on biological monitoring.

7. Federal programs in aquaculture should be maintained at present levels of budgets and manpower in anticipation that economically viable aquaculture will probably develop in the future.

8. To facilitate fisheries resource rehabilitation and aquaculture, a nationally coordinated program is needed on diseases, nutrition, and genetics, accenting cooperation and joint use of facilities by research workers in governments and universities. The need is for improved coordination rather than significant increases in manpower and facilities.

9. Concern is expressed with the relevance and value of much of the federal fisheries resource utilization effort. Improved liaison with, and participation by, industry must be developed. The extension of coastal jurisdiction to 200 miles should provide increased opportunities for resource utilization. This will require additional research, much of which should be done by industry. Meanwhile, the federal manpower devoted to resource utilization should not be increased.

Ways and Means

1. Annual federal operating expenditures in fisheries and marine research should increase in constant dollars from approximately 40 million dollars in 1976 to 60 million dollars by 1981, with a high proportion for shelf-seas research. This increased investment can be justified by the anticipated increase in shelf seas revenue from renewable resources, particularly in the light of extended jurisdiction. The total annual wholesale value of these resources has the potential of reaching 1000 million constant dollars by 1985. The potential production of hydrocarbons in the arctic and continental shelf areas adds further justification for increased research.

2. Long-term research is a necessary base for effective management and should not be sacrificed for short-term needs. To ensure maintenance of the continuity and insulation necessary for research there is need for a separate federal identity for research on fisheries and aquatic resources. In addition, there should be systematic annual scientific evaluations of research programs in federal laboratories, supplemented by independent reviews at intervals of 4–5 years.

3. The future fisheries and aquatic scientists of Canada should be produced in Canadian universities. Opportunities to meet specialized manpower requirements 10 years hence should be patterned on the successful development of oceanography in Canadian universities: a few centers of scientific strength should be identified for special federal support of fisheries and aquatic research, including operating grants, research agreements (subventions), contracts, sharing of capital facilities, and intersessional student employment. Only 1% of the Fisheries and Marine Service research budget is allocated to subventions. This should be increased to 5% and administered jointly with the Environmental Management Service subvention program on inland waters. It should be clearly recognized that the programs of the national granting councils are not sufficiently mission-oriented to ensure adequate support of universities in meeting the requirements of federal government departments.

4. The effectiveness of fisheries and aquatic science programs would be improved if there were less emphasis on species and disciplines and a greater accent on interdisciplinary studies of aquatic ecosystems and their rational use by man. Eminence in the science of marine and freshwater systems would facilitate capitalization on Canada's immense opportunities for rational use of its waters.

5. Too high a proportion of the national program in fisheries and aquatic research is carried out by federal laboratories. Better use of national expertise and facilities would result if provinces, universities, and industry were allotted greater shares of the total activity. Provincial research organizations have competence in areas such as environmental quality and resource utilization; universities have a capability in both natural and social sciences; and industry has a responsibility to increasingly support fisheries and aquatic sciences as part of a user-pays principle.

6. There is need for greater collaboration in fisheries and aquatic sciences in order to break down the barriers prevalent between disciplines and organization units in governments and in universities. This could be achieved through collaborative national and regional arrangements to channel natural and social science expertise to major aquatic resource and environment problems.

7. There is a need to establish criteria for Canadian involvement in international activities in fisheries and aquatic sciences and to evaluate the effectiveness of this involvement in terms of Canadian and global interests. International communication, cooperation, and collaboration are

crucial to fisheries and aquatic sciences. The benefits far outweigh the costs.

8. There is a need for improved communication of scientific and technical information (STI) on fisheries and aquatic sciences to diverse users. This could be brought about by: (a) establishing a Canadian aquatic sciences and fisheries information system, with an analysis and interpretation function, linked to related national and international networks; (b) publishing an applied periodical that interprets developments in fisheries and aquatic sciences and presents these in a form understandable by industry, managers, and other users; (c) developing a professional extension function to transfer science and technology to clients; and (d) enhancing public knowledge of the accomplishments and problems of fisheries and aquatic sciences.

Appendix I

FRB Composition of Committees[2]

Study Committees

Aquatic Renewable Resource Management
Larkin (Chairman 1973–76)
Harding (1974–76)
Russell (1973–76)
Sinclair (1973–76)
Pierce (1973)

Aquatic Environmental Quality
Berlinguet (Chairman 1975–76)
Connor (1975–76)
McMillan (1975–76)
Chant (Chairman 1973–74)
Favre (1973–74)
Geen (1974)
Logie (1973–74)
Omstead (1973)
ex officio (1975)
I. K. Fox
R. R. Logie
K. H. Mann
L. Rousseau
J. B. Sprague

Aquatic Renewable Resource Utilization
Trussell (Chairman 1973–76)
Idler (1973–76)
Langlands (1974–76)
McMillan (1974–75)
Common (1973)
Morrow (1973)

Oceanography
Needler (Chairman 1973–76)
Blanchard (1974–76)
Kendall (1974–76)
Ronald (1973–76)
Filteau (1973)
Harrison (1973)
ex officio (1975)
L. M. Dickie
M. J. Dunbar
G. L. Pickard

Standing Committees

Priorities
Weir (Chairman 1973–76)
Berlinguet (1975–76)
Connor (1975–76)
Kendall (1974–76)
Langlands (1974–76)
Larkin (1973–76)
Needler (1973–76)
Trussell (1973–76)
Chant (1973–74)
Workshop (Sept. 1976)
R. J. H. Beverton
P. Bourgault
H. Favre
I. McTaggart-Cowan
J. K. Reynolds

Scientific Resources
Connor (Chairman 1975–76)
Sinclair (1975–76)
Ronald (1974–76)
Russell (1974–76)
Uffen (1975–76)

Scientific Publications and Information
Idler (Chairman 1974–76)
Blanchard (1975–76)
Brechin (1975–76)
Harding (1975–76)
Sinclair (1974)

[2]The positions and addresses of Board members may be found in the *Fisheries Research Board Annual Report* for the years 1973–76.

Appendix II

FRB Background Studies

No. 1	Science for Canada's Shelf-Seas Fisheries H. A. REGIER and F. D. MCCRACKEN	FRB Manuscript Feb. 1975 Journal Fisheries Research Board of Canada Vol. 32(10) Oct. 1975 Report No. 3
No. 2	Research and Development Related to Utilization of Renewable Aquatic Resources P. A. LEWELL	FRB Manuscript Jan. 1975
No. 3	Biological Oceanography in Canada: Perspective and Review T. R. PARSONS	FRB Manuscript June 1975 Journal Fisheries Research Board of Canada Vol. 32(11) Nov. 1975 Report No. 4
No. 4	Physical Oceanography in Canada C. R. MANN	FRB Manuscript Sept. 1975 Journal Fisheries Research Board of Canada Vol. 33(6) June 1976 Report No. 8
No. 5	Chemical Oceanography in Canada C. R. MANN	FRB Manuscript Sept. 1975 Journal Fisheries Research Board of Canada Vol. 33(6) June 1976 Report No. 9
No. 6	Structured Aquaculture Development with a Canadian Perspective G. I. PRITCHARD	FRB Manuscript Nov. 1975 Journal Fisheries Research Board of Canada Vol. 33(4, 1) April 1976 Report No. 6
No. 7	Science for the Scattered Fisheries of the Canadian Interior H. A. REGIER	FRB Manuscript Dec. 1975 Journal Fisheries Research Board of Canada Vol. 33(5) May 1976 Report No. 7
No. 8	Science for Canada's Atlantic Inshore Seas Fisheries F. D. MCCRACKEN and R. D. S. MACDONALD	FRB Manuscript March 1976 Journal Fisheries Research Board of Canada Vol. 33(9) Sept. 1976 Report No. 11
No. 9	Science for Canada's Fisheries Rehabilitation Needs K. H. LOFTUS	FRB Manuscript March 1976 Journal Fisheries Research Board of Canada Vol. 33(8) Aug. 1976 Report No. 10
No. 10	Aquatic Environmental Quality: Toxicology R. D. HAMILTON	FRB Manuscript March 1976 Journal Fisheries Research Board of Canada Vol. 33(11) Nov. 1976 Report No. 13
No. 11	Aquatic Environmental Quality: Problems and Proposals H. H. HARVEY	FRB Manuscript March 1976 Journal Fisheries Research Board of Canada Vol. 33(11) Nov. 1976 Report No. 12
No. 12	Scientific and Technical Information in Relation to Aquatic Sciences and Fisheries in Canada J. R. WHITEHEAD and I. W. THOMPSON	FRB Manuscript March 1976

No. 12a	An Aquatic Sciences and Fisheries Information Center J. R. WHITEHEAD and I. W. THOMPSON	FRB Manuscript Nov. 1976
No. 13	Scientific Resources for Fisheries and Aquatic Sciences in Canada: The Present Federal Situation P. M. CAISSIE and J. S. WILLMER	FRB Manuscript Sept. 1976
No. 14	Towards a Relevant Science: Fisheries and Aquatic Scientific Resource Needs in Canada H. F. FLETCHER	FRB Manuscript Nov. 1976 Journal Fisheries Research Board of Canada Vol. (In press) Report No. 14
No. 14a	Transdisciplinary Research Centers Oriented to Regional Fisheries Problems H. A. REGIER	FRB Manuscript Nov. 1976

Appendix III

FRB Reports[3]

Aquatic Renewable Resource Management

Science for Management of the Fisheries Resources: An Overview

- Scientific and Technological Needs for the Canadian Shelf-Seas Fisheries
- Science for Canada's Atlantic Inshore Seas Fisheries
- Science for Canada's Fisheries Rehabilitation Needs
- Science for Scattered Fisheries of the Canadian Interior
- Aquaculture in Canada

Oceanography

Oceanography in Canada

Aquatic Environmental Quality

Aquatic Environmental Quality in Canada

Aquatic Renewable Resource Utilization

Research and Development in Canada for the Utilization of Renewable Aquatic Resources

Scientific Resources

Resources for Fisheries and Aquatic Sciences in Canada

Scientific Publications and Information

Scientific and Technical Information in Relation to Aquatic Sciences and Fisheries in Canada

[3]Distributed as Interim Board Reports and to be published as Appendices to the Overview in Report No. 15 in the bilingual report series.

Temperature Preference Studies in Environmental Impact Assessments: An Overview with Procedural Recommendations

The proceedings of a Symposium and Panel Discussion held at the Northeast Fish and Wildlife Conference (Northeast Division, American Fisheries Society), Hershey, Pa., on April 27, 1976

EDITED BY F. P. RICHARDS, W. W. REYNOLDS, AND R. W. MCCAULEY

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Laboratory methods for determining temperature preference	**R. W. McCauley**	749–752
PANEL DISCUSSION		752–754
RECOMMENDATIONS		754–755
REFERENCES		755–761

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Introduction

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CONCERN over waste-heat disposal into United States waterways began in earnest in the mid-1960s. Since then the task of environmental impact assessment has become increasingly important, especially with passage of the Federal Water Pollution Control Act Amendments of 1972. Under these amendments, dischargers of thermal effluents may attempt to demonstrate through the provisions of Section 316(a) that their effluents have no appreciable detrimental impact on the aquatic biota.

In Canada most of the concern over waste-heat discharge began in the early 1970s when the federal Department of the Environment was formed. But as early as 1968 the Ontario Water Resources Commission (now the Ontario Ministry of the Environment) had instituted studies on thermal inputs to the Great Lakes. The enactment of the Canada Water Act resulted in federal–provincial water quality cooperation. Since natural resource ownership in Canada is basically provincial, each province has established its own guidelines to ensure water quality (A. Johnson, Ontario Ministry of the Environment, personal communication).

In many cases, environmental impact predictions must be based to a greater or lesser extent on previously published data. These predictions would be greatly facilitated by some degree of methodological standardization, if such could be agreed upon. This was one goal of the present symposium. By examining critically the history of temperature preference studies and methodologies, comparing the results, and assessing the present technology, we hoped to distill the best of our collective knowledge for use in untying the knot of behavioral responses of fishes to temperature. This symposium was intended to serve as a primer for those unfamiliar with temperature preference studies and as a source of up-to-date information for those actively working in this area of research.

The five formal papers cover the following basic areas: physiological responses to changing temperature, theoretical background of behavioral responses to temperature, a tabulation of published results, application of these results to impact assessments, and a review of laboratory apparatuses and methodologies. Bibliographies have been collated to eliminate redundancy and to provide easy reference for other workers with similar interests.

After the presentation of papers, an hour-long, open panel discussion was held during which various concerns and opinions regarding temperature preference studies were aired. These discussions were followed by a meeting of the six symposium members at which time general recommendations for the future conduct of temperature preference studies were proposed.

We hope that these efforts will generate further inspiration and discussion among researchers regarding techniques and interpretation of temperature preference studies.

I thank P. V. Morgan and B. C. Marcy of NUS Corporation for their initial encouragement in my organization of this symposium. Ecological Analysts, Inc., provided financial support. I also thank all the panel members, especially W. W. Reynolds and R. W. McCauley, who helped me to edit these symposium proceedings.

Physiological and Behavioral Reactions of Fishes to Temperature Change

L. I. CRAWSHAW[1]

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CRAWSHAW, L. I. 1977. Physiological and behavioral reactions of fishes to temperature change. J. Fish. Res. Board Can. 34: 730–734.

Teleost fishes possess a central nervous system thermoregulatory mechanism remarkably similar to that of other vertebrates. Inputs from peripheral and anterior brainstem thermosensitive elements are integrated to effect appropriate thermoregulatory responses. The integrated output signal from the thermoregulatory center also appears to provide an input to the respiratory system. Short-term deviations from a given temperature alter respiratory requirements, produce acid–base imbalance, and cause disturbances in fluid–electrolyte regulation. Acclimation to a given temperature involves changes that counteract these disturbances.

Key words: fish, temperature change, behavioral responses, physiological responses, temperature regulation

CRAWSHAW, L. I. 1977. Physiological and behavioral reactions of fishes to temperature change. J. Fish. Res. Board Can. 34: 730–734.

Les téléostéens possèdent un mécanisme thermorégulateur dans le système nerveux central remarquablement semblable à celui des autres vertébrés. Les sensations provenant d'éléments thermosensibles périphériques et antérieurs du cerveau sont intégrées de façon à produire des réponses thermorégulatrices appropriées. Le signal de sortie intégré provenant du centre thermorégulateur semble également fournir mtune entrée vers le système respiratoire. Des écarts de courte durée à partir d'une température donnée ont pour effet d'altérer les exigences respiratoires, de produire un déséquilibre acide–base et de causer des perturbations dans le réglage fluide–électrolytes. L'acclimatation à une température donnée comporte des changements qui contrebalancent ces perturbations.

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FISHES inhabiting the temperate zone often encounter relatively large temperature changes, over short (minutes and hours) and long (seasonal) time spans, which necessitate appropriate behavioral and physiological adjustments. The importance of these adjustments, and the extent to which they are controlled by the central nervous system are the central topics of this paper.

Physiological Temperature Optima

Temperatures selected by fishes very likely represent temperatures at which they have evolved to carry out physiological functions with maximal efficiency. Under certain conditions physiological processes may have different thermal optima, and this may result in changes in the preferred temperature. For example, Brett (1971) found that sockeye salmon (*Oncorhynchus nerka*) normally select 15°C, a temperature at which active metabolism, sustained swimming speed, and cardiac work are maximal. With unlimited food rations, young salmon sustain optimum growth at this temperature. However, if rations are limited, growth optima occur at progressively lower temperatures (Brett et al. 1969; Brett and Higgs 1970), a fact which Brett (1971) used to explain diurnal migration of sockeye salmon smolts into deep, cold water. Graham (1949) and Fisher and Elson (1950) have also demonstrated the coincidence of various physiological optima with selected temperature in other salmonids. The actual temperature selected and the concomitant physiological optima among teleosts vary widely. Extremes are demonstrated by desert pupfish (*Cyprinodon macularius*), which when active during the summer select water up to 40°C (Lowe and Heath 1969), and Antarctic icefish (*Chaenocephalus aceratus*), which exit rapidly from water warmed to 5°C (Crawshaw and Hammel 1971).

The functions relating various physiological optima with acclimation temperature often depict a zone of efficient operation rather than a sharp peak, reflecting the ability of fishes to adjust internal conditions to different thermal environments (acclimation). However, once a fish has

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been at a particular temperature for a period of time, even rather small temperature changes lead to major shifts in metabolism, fluid–electrolyte balance, and acid–base relationships; this helps to explain the value of behavioral homeostasis (temperature preference).

Acute Effects of Temperature Change

The effect of temperature on metabolic rate necessitates major, acute cardiovascular and respiratory adjustments following sudden changes in water temperature. Meuwis and Heuts (1957) found that the respiratory frequency of carp (*Cyprinus carpio*) was doubled by a temperature increase from 32 to 36°C. After 48 h sufficient thermal acclimation had occurred that the frequency decreased to only 15% above the original level. Fry (1947) cautioned that increases in activity and level of arousal may account for some of the initial respiratory increase following transfer to higher temperature. Overshoot and hysteresis phenomena have also been noted in cardiovascular and respiratory frequencies during heating and cooling in largemouth blackbass, *Micropterus salmoides* (Reynolds 1977b).

Since the gills are of primary importance in fluid–electrolyte balance, increases in ventilation and perfusion increase the cost of maintaining this balance. Changes in temperature per se are also disruptive of fluid–electrolyte balance, since "both the absolute magnitude and the rate of application of the imposed stress seem to be as critical, in terms of their effects, as the direction in which the temperature shift takes place" (Houston 1973). Fishes, like other vertebrates, appear to regulate the blood at a constant relative alkalinity rather than at a constant pH (Howell et al. 1970; Rahn and Baumgardner 1972; Randall and Cameron 1973). When fish undergo a temperature change, either arterial plasma carbon dioxide (P_{CO_2}) or plasma bicarbonate concentration has to be adjusted to maintain a constant relative alkalinity. Randall and Cameron (1973) have shown that the latter process is utilized and takes place over a period of hours. After a rapid temperature change, then, fish experience a transient acid–base imbalance.

A Control Model

There appears to be an underlying similarity between the central nervous system (CNS) mechanisms regulating body temperature in all vertebrates. Endotherms (mammals and birds) use both physiological (autonomic) and behavioral responses to maintain a constant internal temperature. They tend to avoid temperatures above or below their thermoneutral zone; failing this, they activate physiological responses such as shivering or panting which, although costly in terms of energy or fluid balance, maintain a constant internal temperature. Both physiological and behavioral thermoregulatory mechanisms are similarly controlled in that peripheral and anterior brainstem temperatures are integrated to produce a restorative response of appropriate direction and magnitude (Stitt et al. 1971). Both the rate of change of peripheral temperature and the absolute peripheral temperature contribute inputs to the thermoregulatory center (Cabanac 1975), while only the absolute temperature of the anterior brainstem appears important (Hammel 1968).

There are indications that behavioral temperature regulation is similar in fish and mammals, in terms of accuracy and underlying control mechanisms (Hammel et al. 1973). This is not true for the physiological responses. Aquatic gill breathing precludes the maintenance of an internal body temperature much above ambient levels (Stevens and Fry 1970, 1974). Exceptions to this rule include only tunas and lamnid sharks, which have evolved specialized anatomical adaptations for countercurrent heat exchange to conserve heat in the lateral swimming muscles (Carey 1973).

Although the thermoregulatory centers in a fish cannot help provide a constant internal body temperature, they might serve to anticipate physiological changes which inevitably accompany thermal change. Such responses would enable the animal to initiate restorative responses before a deficit in the regulated variable could occur. Fishes probably possess such a system; indeed, it may be just this system which later evolved into the physiological homeothermic control system utilized by endotherms.

A hypothetical model incorporating these ideas is presented in Fig. 1. The two central boxes refer to CNS structures (probably in the anterior brainstem) where incoming thermal information is integrated and compared with a hypothetical "reference temperature." Appropriate restorative (behavioral) and anticipatory (respiratory) responses are then activated. The extent to which this system participates in the activation of compensatory responses (acclimation) is unclear. The dashed lines depict flows of information, while the solid lines indicate significant involvement of energy. When a fish encounters changing water temperatures, the temperature at the general body surface and buccal cavity is rapidly altered. The rate of change and absolute temperature are im-

portant in determining informational input from these areas. The temperature change also initiates heat transfer to more internal portions of the body, both at the gills and through the general skin surface. The ensuing changes in deep body temperatures are then also sensed. Information from peripheral and central thermosensitive sites are integrated and compared with the reference temperature. If these inputs indicate a deviation from the reference temperature, appropriate behavioral and physiological responses are initiated. Thus, when a fish enters water that is above its reference temperature, peripheral (and soon thereafter central) temperature transducers initiate inputs which are integrated and compared with the reference temperature. Appropriate responses include the seeking of cooler water and the initiation of increased respiratory activity. Large-scale disturbances of almost any sort initiate a CNS arousal mechanism that inhibits or overrides behavioral and physiological response to thermal inputs (such input to the respiratory control centers was left out of the diagram to avoid obscuring the major relationships). The reference temperature apparently is shifted in some cases by the season (Sullivan and Fisher 1953; Barans and Tubb 1973), time of day (Barlow 1958; Brett 1971; Reynolds and Casterlin 1976a), nutritional status (Javaid and Anderson 1967b), bacterial pyrogens (Reynolds et al. 1976a; Reynolds 1977c; Reynolds and Covert 1977), and anaesthetics (Ogilvie and Fryer 1971). The interrelationship between thermal acclimation and the thermoregulatory centers is somewhat ambiguous.

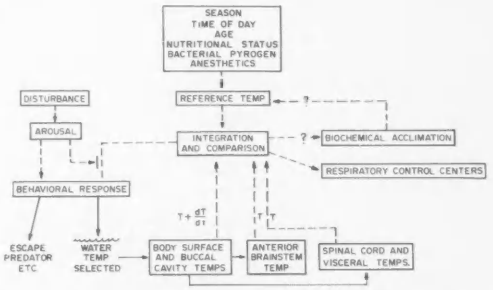

FIG. 1. A scheme depicting how CNS mechanisms enable teleost fishes to cope with variations in the thermal environment. Dashed lines depict flows of information, while solid lines indicate the involvement of significant amounts of energy. The three central boxes (reference temperature, integration and comparison, and arousal) refer to processes occurring in the central nervous system.

Effects of Peripheral Temperature Change

Two important events are initiated by changing water temperatures: (1) transmission of peripheral thermal information to the CNS and (2) thermal exchange with the altered environment. Fish apparently possess a diffuse cutaneous sensitivity to temperature. Bardach (1956) trained goldfish (*Carassius auratus*) to swim away when touched by a warmed rod and not to move when the rod was at ambient temperature. Responses were not obtained until the area of the tip of the rod was increased to about 2 mm^2 and the temperature was at least 2°C warmer than ambient, at which point responses were obtained over almost the entire body surface, including the fins. Dizon et al. (1974) studied classical heart rate conditioning in skipjack tuna (*Euthynnus pelamis*) using temperature as the conditioned stimulus. They found that thermal stimuli were more effective when delivered to the oral-branchial cavity than when delivered to the dorsal anterior quadrant of the body surface. This indicates that peripheral temperature-sensitive neurons either occur in greater density in the oral-branchial cavity or receive heavier weighting when processed by the CNS. Murray (1971) reported whole-body temperature sensitivities of fishes ranging from 0.1 to 0.03°C. The receptors involved have not yet been definitively identified, but the importance of peripheral input to behavioral thermoregulation in fishes is provided by cocaine-desensitization experiments by Sullivan and Fisher (reported by Sullivan 1954) and also by escape latency experiments by Crawshaw and Hammel (1971).

Thermal Exchange with the Environment

Thermal exchange between fish and their environment has been investigated by Kuroki (1967), Ihssen (in Fry 1967), Stevens and Fry (1970, 1974), Müller (1974), Spigarelli et al. (1974), Stauffer et al. (1975c), Crawshaw (1976), and Reynolds (1977b). The rate of thermal equilibration following an ambient temperature change is affected by such factors as rates of gill ventilation and blood flow, circulatory anatomy, activity, water movement, and shape and size of the fish.

Among the most important factors a fish has to cope with following a rapid temperature change is altered respiratory demand. The best means of estimating a temperature-induced change in respiratory requirements would be to measure mean body temperature ($\bar{T}_b$). Crawshaw (1976) estimated the relationship between $\bar{T}_b$ and deep body-core temperatures. Immediately after

a rapid ambient temperature change, dorsal muscle temperature changes much more slowly than $\overline{T}_b$. Brain temperature changes slightly faster than dorsal muscle temperature, while intestine temperature changes more slowly.

Central Thermal Inputs

As deep body temperatures begin to shift, information from these sites is added to that coming from the periphery. Sullivan (1954) noted that destruction of the forebrain abolished normal responses to temperature. Heating and cooling of the rostral forebrain with implanted thermodes (Hammel et al. 1969; Crawshaw and Hammel 1971, 1974) altered temperature responses, demonstrating the effect of central temperature-sensitive elements. Greer and Gardner (1970, 1974) recorded extracellular action potentials from temperature-sensitive neurons in the brains of brook trout (*Salvelinus fontinalis*) and found changes in firing rate following temperature changes induced by lowering the temperature of water irrigating the gills. One neuron was found which decreased its firing rate during cooling of the mouth and gills.

Respiratory Homeostasis During Temperature Change

Assuming that changes in $\overline{T}_b$ provide a reasonable estimate of the rapidity with which changes in water temperature alter respiratory requirements, a fish moving in a heterogeneous thermal environment will encounter very rapidly changing respiratory demands. The teleost respiratory system as conventionally depicted (Shelton 1970; Heath 1973) could cope with this situation, since thermally induced increases in metabolic rate would lead to decreases in dorsal aortic P_{O_2} ($P_{a_{O_2}}$). Decreases in $P_{a_{O_2}}$ are rapidly sensed by fish and lead to a compensatory increase in respiratory activity (Shelton 1970; Saunders and Sutterlin 1971; Bamford 1974). However, maintenance of $P_{a_{O_2}}$ is of paramount importance to fish, and utilization of a respiratory control system sensing only P_{O_2} would cause fluctuations in $P_{a_{O_2}}$ in heterogeneous thermal environments and implies that $P_{a_{O_2}}$ would decrease as the temperature of the fish increased (this would be necessary to provide an error signal for the required increases in ventilation and perfusion of the gills). If the respiratory centers in the medulla were to receive inputs from the temperature-sensitive areas in the periphery and anterior brainstem, changes in P_{O_2} could be anticipated and $P_{a_{O_2}}$ could be kept relatively constant. There exists an analogous system in fish that regulates $P_{a_{O_2}}$ during exercise. In this case, the input is probably from proprioceptors, since increases in activity lead to extremely rapid respiratory adjustments (Stevens and Randall 1967; Sutterlin 1969).

Anterior brainstem temperature influences gill ventilation (Crawshaw et al. 1973). Changes in gill ventilation ($\dot{V}_G$) are related to the absolute temperature, not to the rate of change. Heating or cooling portions of the brain anterior or posterior to the anterior brainstem was not effective in altering $\dot{V}_G$. Randall and Cameron (1973) found that several hours after a temperature increase from 10.5 to 20.0°C, $\dot{V}_G$ and oxygen consumption in rainbow trout (*Salmo gairdneri*) were markedly increased, although $P_{a_{O_2}}$ did not change. Iriki et al. (1976) found that heating and cooling the spinal cords of *Cyprinus carassius* and *C. carpio* led to changes in heart rate, indicating the existence of spinal thermosensitive elements that influence the teleost cardiovascular system.

Demonstrating peripheral thermal input to anticipatory respiratory control mechanisms is difficult, since a temperature change rapidly alters metabolic requirements. An increase in ambient water temperature causes a rapid, monotonic increase in $\overline{T}_b$. The respiratory requirements thereby induced should presumably follow a similar course, deviations from which could indicate another input. Figure 2 (from Crawshaw 1976) shows the mean change in $\dot{V}_G$ following a rapid shift in the temperature of the inspired water. The water was saturated with air after the temperature was shifted. Note the rate-sensitive nature of the response, which could be due to input from peripheral temperature-sensitive neurons. In other experiments, rapid decreases in inspired water temperature produced rate-sensi-

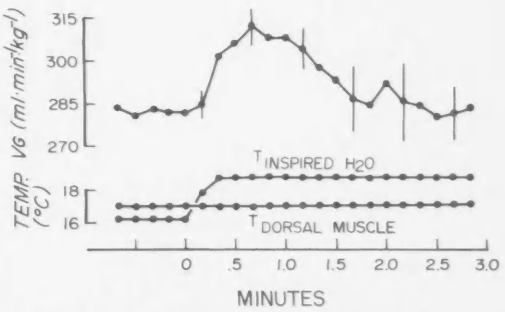

FIG. 2. Mean changes in gill ventilation ($\dot{V}_G$) following repeated increases in the inspired water temperature for a 0.85-kg carp (*Cyprinus carpio*). From Crawshaw (1976).

tive decreases in $\dot{V}_G$. Changing the temperature at the body surface (exclusive of the gills) produced similar rate-sensitive increases and decreases in $\dot{V}_G$ but of a lesser magnitude.

Thermal Acclimation

As acute physiological responses are mobilized to meet the demands caused by changing ambient temperature, compensatory acclimation responses are initiated which enable the animal to operate more efficiently at the new temperature over the long term. This process occurs over two distinct time spans. Some adjustments are relatively rapid and are termed instantaneous temperature compensation. Such mechanisms are highly developed in intertidal invertebrates (Newell 1969) and are likely present to some extent in fishes. Hazel and Prosser (1974) discuss many modes of operation of instantaneous thermal compensation; examples include thermal effects on enzyme–substrate interactions and thermally induced alterations in enzyme structure. These changes operate to counteract the usual effects of temperatures on chemical reactions and can thus provide a degree of temperature independence. Much of the thermal acclimation process in fish occurs over a span of hours and days, and involves a "biophysical and biochemical restructuring of many cellular and tissue components for operation under the new thermal regime imposed on the organism" (Fry and Hochachka 1970). These changes apparently involve "feedback to the genetic material . . . and subsequently to the protein synthetic system" (Hazel and Prosser 1974).

As a result of changes induced by thermal acclimation, fish exhibit altered thermal optima for various physiological activities and tend to select temperatures accordingly. Although the CNS itself undergoes acclimation and thereby exerts an important influence over the rest of the organism (Lagerspetz 1974), the involvement of the CNS thermoregulatory system in the initiation of thermal acclimation is unclear at present. The importance of brain temperature was shown by Precht (1961), who acclimated the anterior and posterior ends of eels to different temperatures and found that overall oxygen consumption corresponded to that predicted by the temperature of the head. Schultze (1964) confirmed Precht's results but also found that the respiratory activity of isolated muscle slices was characteristic of the temperature to which they were acclimated. Fahmy (1972, 1973) performed similar experiments on brook trout, using time to death at a lethal temperature as the dependent variable. He concluded that "the tissue exposed responds according to its individual level of thermal acclimation."

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Temperature as a Proximate Factor in Orientation Behavior

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Temperature serves as a proximate factor (cue, guidepost, sign stimulus, or directive factor) affecting locomotor responses of fishes. Although temperature can also serve as an ultimate ecological factor, as in behavioral thermoregulation, nonthermal factors may in some cases provide the ultimate adaptive or ecological value of a temperature response; some examples are habitat selection, intraspecific size segregation, interspecific niche differentiation, isolating mechanisms, predator avoidance, prey location, escape reactions, and migrations (thermoperiodic, diel, seasonal, spawning). Conversely, nonthermal variables such as light intensity

or water depth may act as accessory proximate factors in thermoregulation. In spawning migrations, thermal requirements of eggs and larvae may take precedence over the (often different) preferenda or optima of adults. Although thermal responses of fishes are largely innate and species specific, ontogenetic and other changes can occur. Since temperature can serve as an unconditioned reinforcer in operant conditioning, thermal responses are not limited to simple kineses or taxes. Nonthermal factors such as photoperiod, circadian rhythms, currents, social and biotic interactions, stresses, infections, or chemicals can affect thermal responses, and may account for some lack of conformity between laboratory preferenda and field distributions and behaviors.

Key words: thermoregulation, orientation, preferendum, selection, preference, avoidance, behavior, temperature, fish, responses

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La température sert de facteur initial (indicateur, guide, stimulus ou facteur directeur) affectant les réponses locomotrices des poissons. Bien que la température puisse également servir comme facteur écologique ultime, comme dans le cas de la thermorégulation de comportement, des facteurs non thermiques peuvent dans certains cas fournir la valeur adaptive ou écologique ultime d'une réponse à la température; comme exemples, on peut mentionner le choix de l'habitat, la ségrégation intraspécifique selon la taille, la différenciation interspécifique des niches, les mécanismes d'isolement, l'évitement des prédateurs, la localisation des proies, les réactions d'échappement et les migrations (thermopériodiques, nycthémérales, saisonnières, reproductrices). Inversement, des variables non thermiques telles que l'intensité lumineuse ou la profondeur d'eau peuvent agir comme facteur initial accessoire dans la thermorégulation. Dans les migrations de fraie, les exigences thermiques des œufs et des larves peuvent avoir préséance sur les températures préférées ou optimales (souvent différentes) des adultes. Bien que les réponses thermiques des poissons soient en grande partie innées et particulières à l'espèce, il peut se produire des changements ontogéniques ou autres. Etant donné que la température peut servir de renforcisseur non conditionné dans le conditionnement opérant, les réponses thermiques ne sont pas limitées à de simples cinétiques ou tactismes. Des facteurs non thermiques tels que la photopériode, les rythmes circadiens, les courants, les interactions sociales et biotiques, le stress, les infections ou les produits chimiques peuvent affecter les réponses thermiques et expliquer le manque de conformité entre les températures préférées observées en laboratoire et la répartition et le comportement sur le terrain.

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SINCE temperature is a major factor affecting the life processes of organisms, it is not surprising that mobile organisms, including fishes, exhibit locomotor responses to thermal stimuli. In contrast to the passive habitat correlation of sessile organisms with temperature, largely reflecting differential survival, a mobile organism typically expresses active temperature preference or avoidance responses that enhance or optimize its chances of survival and reproduction, thereby contributing to its evolutionary fitness.

Preference and Avoidance

The terms "selected" or "preferred" temperature, or "thermal preferendum," are defined operationally as the range of temperatures in which animals congregate or spend the most time in a gradient or free-choice situation. The most meaningful single characteristic of a species' thermal behavior is the "final preferendum," defined by Fry (1947) as that temperature toward which a fish will finally gravitate regardless of its previous thermal history and where preferred and acclimation temperatures are equal. If a fish is left in a thermal gradient for a sufficient length of time, it will eventually gravitate and become acclimated to its final preferendum.

Frequently the distribution of selected temperatures is skewed (Ivlev and Leizerovich 1960; DeWitt 1967; Reynolds and Casterlin 1976a), so several statistics, or preferably the entire frequency distribution, should be given to fully characterize the temperature response of an organism (Evropeyzeva 1944; Ivlev 1958; Ivlev and Leizerovich 1960; Reynolds and Casterlin 1976a; V. H. Hutchison, University of Oklahoma, personal communication).

Where a unimodal response is evident, the mode or other measure of central tendency emphasizes the positive or preference aspect of the behavior. The mode corresponds to the definition of preferred temperature as that temperature occupied most frequently (Pitt et al. 1956;

Ivlev and Leizerovich 1960), thus the term "modal preferendum" (Reynolds and Thomson 1974a; Reynolds and Casterlin 1976a; Reynolds et al. 1977). This statistic is easily determined by inspection of the frequency distribution and serves best in cases of skewed distributions, indicating diel or seasonal differences even with little or no accompanying change in the mean, range or midpoint (DeVlaming 1971; Reynolds and Casterlin 1976a). DeWitt (1967) has hypothesized that skewness may be due to a nonlinear effect of temperature on metabolism, but Reynolds and Casterlin (1976a) have shown that skewness may be either positive or negative, or absent, in some species depending on the time of day or night. The strength or precision of the active "seeking" tendency is characterized by the degree of kurtosis in the distribution curve (Ivlev and Leizerovich 1960). The modal preferendum might be compared to a specific "search image," or characterized by a hypothalamic "set-point" (Hammel 1968).

The negative aspect of thermal behavior is termed "avoidance," most overtly characterized by escape or aversive reactions elicited by non-preferred temperature extremes often called upper and lower "turnaround" or avoidance temperatures (Neill and Magnuson 1974; Beitinger 1974). This aspect is most evident in distributions characterized by a broad plateau which drops off steeply on both sides or which is bi- or polymodal, with no obvious central tendency as in a unimodal distribution. Here one is again faced with a somewhat arbitrary choice of statistics. It is necessary to state clearly in each instance whether "avoidance temperatures" refers to temperatures never voluntarily occupied or to temperatures seldom occupied; if the latter, "seldom" must be clearly defined in statistical terms (Reynolds and Casterlin 1976a; Meldrim and Gift 1971; Barans and Tubb 1973; Beitinger 1974; Neill and Magnuson 1974). The distribution of voluntarily occupied temperatures largely determines our subjective emphasis on the positive (preference) vs. the negative (avoidance) aspects of thermal behavior.

We are faced with the question of whether a fish at a given moment is actively seeking out a preferred temperature, or is merely avoiding aversive temperatures. Here there would seem to be a difference between actively swimming species, and more sedentary benthic or "hovering" species. The latter are refractory and appear reluctant to move, and only do so in response to aversive stimuli such as extreme temperatures (DeVlaming 1971), while active species such as centrarchids are characterized by a more positive preference response (Reynolds and Casterlin 1976a).

A further answer to the preference–avoidance question may hinge upon the extent to which the central neural mechanism controlling behavioral thermoregulatory responses is either proportional or "on–off" in nature. This central mechanism is currently thought to be homologous among all vertebrates (cf. Crawshaw and Hammel 1973; Vaughn et al. 1974; Reynolds et al. 1976a), but the exact nature of its functioning is not yet fully understood. Hammel (1968) states that "internal temperature is compared in some way with a reference, and when a difference occurs an appropriate response is made tending to reduce but not eliminate that difference" (p. 641), and "there is increasing evidence that the dominant type of control possessed by the hypothalamic controller is proportional for at least some of the physiological responses. Proportional control means that the regulatory response is proportional to the difference between the hypothalamic temperature and a threshold or set temperature" (p. 686). We might reasonably infer that the reference or set-point temperature postulated by Hammel may correspond to the preferred temperature of ectotherms, and proportional control would tend to yield a unimodal preferred-temperature distribution.

Other workers (e.g. Berk and Heath 1975; Barber and Crawford 1976) have suggested an upper and a lower set-point, implying limit-sensing on–off control reminiscent of upper and lower turnaround or avoidance temperatures. This model also predicts a unimodal preferred-temperature distribution, with skewness depending upon the relative standard deviations of the upper and lower set-points (Barber and Crawford 1977).

Mitchell et al. (1970) have suggested yet another thermoregulatory model which employs no real set-point or reference temperature whatever. They argue that none is necessary and that the concept of a "set-point" as applied to biological systems is purely a mathematical abstraction borrowed from engineering. Their model depends upon counterbalanced negative feedback from both warm and cold sensors. It does not yet appear possible to make a definitive choice among these competing models.

Behavioral Mechanisms

Fraenkel and Gunn (1961) have classified orientation responses of invertebrates into simple taxes and kineses. Some investigators have attempted to relate movements of fishes to simple

kinetic effects of temperature (Shaw et al. 1938; Fisher and Elson 1940, 1950; Elson 1942; Fisher and Scott 1942; Sullivan and Fisher 1947; Fry and Hart 1948; Graham 1949; Fisher and Sullivan 1958; Ivlev 1960; Peterson and Anderson 1969; Ellgaard et al. 1975), although Sullivan (according to Fry 1964, p. 720) "objects to classifying the temperature selection response in fish as a thermokinesis in the sense of Fraenkel and Gunn on the grounds that the thermal experiences of the fish from moment may be continuously modifying its response in the gradient, a situation by no means within the scope of their definition." Also, the relationships between activity levels of a fish and temperature have been contradictory among various studies, leading to considerable doubt as to the existence of a simple and direct causal relationship between kinetic effects and temperature selection behavior (Fry 1964). Sale (1969) has advanced what is in effect a kinetic argument for habitat selection in general, contending that exploratory or appetitive behavior is higher in "poor" or nonpreferred habitats and is replaced by consummatory or nonexploratory behaviors in more optimal or preferred environments. However, Rozin and Mayer (1961), who conditioned goldfish (*Carassius auratus*) to press a bar to regulate water temperature (temperature thereby serving as an unconditioned reinforcer), add final condemnation to purely kinetic arguments: "It has been suggested (Fisher and Elson 1950) that temperature selection can be accounted for as a direct effect of temperature on the locomotion of fish. This study indicates that other factors are involved in temperature selection, since the goldfish will perform an arbitrary response to change the temperature of its environment." Rozin and Mayer's values were somewhat higher than the final preferendum of about 28°C reported for goldfish in conventional gradients (Fry 1947; Ferguson 1958) or in an electronic shuttlebox (Reynolds and Covert 1977), suggesting that they may have measured upper avoidance temperature.

Recent studies have indicated no direct causal relationship between circadian activity levels and thermoregulation (Beitinger 1975; Beitinger et al. 1975; Crawshaw 1975a; Reynolds and Casterlin 1976b, 1976c; Reynolds 1977a). For example, locomotor activity levels of bluegill (*Lepomis macrochirus*) differ by a factor of 2 between night and day (Reynolds and Casterlin 1976b), with no significant change in mean preferred temperature, range or midpoint, although the mode and skewness change (Reynolds and Casterlin 1976a).

The term "thermotaxis" also seems inappropriate for fish thermal responses. The responses of fish to gradients are seldom of the "all or none" type and therefore lack the precision of classical taxes or forced movements (Viaud 1955; Hoar et al. 1957; Reynolds et al. 1977).

Proximate and Ultimate Factors

When an organism responds behaviorally to thermal stimuli, temperature serves as a "proximate factor" in orientation. It cannot be assumed on a priori grounds that the perceptual basis (proximate factor) for a given response necessarily indicates which environmental variable (ultimate factor) is most important for the direct physiological or ecological well-being of the individual or for its genetic contribution to the continuation of the species (Baker 1971). In the case of behavioral thermoregulation, where control of the animal's body temperature provides the adaptive value of the response, temperature serves as both proximate and ultimate factor. However, the proximate and ultimate factors governing a response need not always be the same, so long as they are sufficiently coupled to provide an overall adaptive value to the response. Nonthermal proximate cues (such as light or water depth) could aid in thermoregulation.

Thermal cues acting as proximate factors may help to guide a migrating fish into a spawning habitat optimal for eggs, larvae, and/or juveniles (Chidester 1924; Fry 1937; Collins 1952; Fleming and Laevastu 1956; Cooper 1961; Foster 1969; Lund et al. 1970; Richkus 1974; Blackburn and Williams 1975), whose requirements may differ from those of the adults (Ivlev and Leizerovich 1960; Brett 1970; Reynolds and Thomson 1974a, b, c; Blackburn and Williams 1975; Reynolds et al. 1976c). The genetic contribution to successive generations is more important for evolution than the longevity of the individual organism per se, so thermoregulatory optima of adult animals would be subordinate during the spawning season to the reproductive requirements. A relative abundance of food or absence of predators or competitors might also serve as ultimate factors, with temperature being the proximate factor (Fleming and Laevastu 1956; Brett 1970; Reynolds and Thomson 1974a; Blackburn and Williams 1975).

The associations between proximate orientation cues and the ultimate ecological factors conferring adaptive value on preference behavior could be either phylogenetically acquired through natural selection acting on inheritable response tendencies, or ontogenetically acquired through learning, or both. The former mechanism is

thought to be of greater importance in fishes (Hoar et al. 1952; Dzyan 1959; Fry 1964; Feddern 1968; Scherer 1971; Goddard and Tait 1976). Inherited thermal preferences would constitute an important part of the species niche, helping to isolate species in space and time (Manning 1967; Scherer 1971), thus playing a role in the evolution and maintenance of species (Rosenblatt 1963). However, ontogenetic behavioral changes (not necessarily due to learning) can also occur (Ivlev and Leizerovich 1960; Norris 1963; McCauley and Read 1973; Reynolds and Thomson 1974a, b).

Laboratory vs. Field Behavior: Fact or Artifact?

A final and crucial question concerns the relationship between laboratory preferenda and the thermal behavior of fishes under natural conditions. In general, thermal preferenda of fishes as determined in the laboratory have proven to be fairly reproducible and species specific, regardless of diverse measures and methods used (cf. Reynolds and Casterlin 1976a). However, numerous nonthermal stimuli affect the distributions of fishes and other ectothermic vertebrates, including intraspecific social interactions such as dominance hierarchies and territoriality (Bacon et al. 1967; DeWitt 1967; Beitinger and Magnuson 1975) and interspecific interactions such as predation, competition, feeding, and habitat selection–niche segregation (Fleming and Laevastu 1956; Brett 1970). Bacterial infection can increase the preferendum (Reynolds et al. 1976a; Reynolds and Covert 1977; Reynolds 1977c). Abiotic factors may also affect thermal responses to a greater or lesser degree (Sullivan and Fisher 1953, 1954; Ferguson 1958; Medioni 1963; Zahn 1963; Ogilvie and Anderson 1965; Meldrim and Gift 1971; Barans and Tubb 1973; Peterson 1973; Reynolds et al. 1977a; Richards 1974).

Different investigators have found varying degrees of relationship between laboratory preferenda and field distributions (Dendy 1948; Ferguson 1958; Gibbons and Bennett 1971; Gibbons et al. 1972; Kelso 1974, 1976; Neill and Magnuson 1974; Reynolds and Thomson 1974a; Stauffer et al. 1974, 1975a, b; Kelso and Minns 1975; Reynolds and Casterlin 1976a; Reynolds et al. 1977). Fishes in nature are often found at temperatures lower than their final preferenda as determined in the laboratory. This can not always be fully attributed to size–age effects (Ferguson 1958; McCauley and Read 1973), since larger adult fish may also show a high laboratory preferendum (Reynolds et al. 1976b). In some cases, the availability of preferred temperatures is limited in time or space. Where sufficiently high temperatures are available, as in thermal effluents, it still appears that individual fish do not consistently remain in preferred-temperature areas indefinitely (Kelso 1974, 1976; Neill and Magnuson 1974; Richards 1974; Kelso and Minns 1975). Of course, even in the laboratory, individual fish will continually move in and out of the modal preferred temperature; however, in a laboratory gradient there is little possibility of the fish becoming distracted or lost from the influence of the thermal gradient.

Where netting or rotenone is used in field-sampling studies (Stauffer et al. 1974, 1975a, b), an artifact might be introduced by a possible effect of temperature on susceptibility to rotenone or ability to escape from an area being netted, leading to the appearance of an inverse correlation between preferred temperature and field distributions for some species (Reynolds and Casterlin 1976a). This could also occur with electroshocking (Neill and Magnuson 1974), but ultrasonic tracking (Kelso 1974, 1976) and acoustic census (Kelso and Minns 1975) may not be similarly biased.

While thermal distributions in nature often lack the precision seen in the laboratory, there are some indications that even laboratory preferenda may become lower and/or less precise after about a week or more in a gradient (Bacon et al. 1967; DeVlaming 1971; Reynolds and Thomson 1974a). The fairly high, precise preferenda measured within the first few days in a laboratory gradient may conceivably reflect the stress of the laboratory situation and may decrease with habituation. Stress can increase the precision of thermoregulatory responses. Regal (1971) found that an isolated lizard would cease operant thermoregulatory behavior after being alone for a period of time, but would resume thermoregulation at a characteristic high temperature when a second lizard (to which it was socially dominant) was introduced. He concluded (p. 4) that "the laboratory environment may not be, in some cases, sufficiently stimulating, in a 'psychological' sense, to cause the lizards to regulate at the higher temperatures that they might regulate at in nature." According to Brett (1956, p. 85), "it would seem that an inherent ability to perceive fine gradients of temperature is only exercised when called upon by some internal drive (possibly in some migrations) or by an environmental stress (possibly at higher temperatures)." There could be a relationship (not necessarily causal) with exploratory behavior, which tends to be higher at first in novel environments and to de-

crease with time (Kleerekoper et al. 1974; W. W. Reynolds unpublished data). The stress of infection, mediated by pyrogens, can increase the preferendum by several degrees (Reynolds et al. 1976a; Reynolds and Covert 1977; Reynolds 1977c), which somehow enhances survival from the infection (J. B. Covert and W. W. Reynolds unpublished data). Stress-enhanced thermoregulation might "fine-tune" the physiological responses of the organism, such as the immune response to infection or escape reactions from harmful stimuli. Fish maintained in laboratory temperature and photoperiod conditions comparable to those of the spawning season may also be in perpetual breeding condition (Banner and Hyatt 1975; Smith 1975), so their preferenda might reflect spawning optima.

Another possible interpretation of initially high laboratory preferenda involves initial overshoot during gravitation to the final preferendum (cf. Badenhuizen 1967; Beitinger and Magnuson 1976), a phenomenon not uncommon in physiological responses to temperature changes (Prosser 1965; Peterson and Anderson 1969; Reynolds 1977b). Alternatively, the laboratory responses may be considered to represent the pure species-specific temperature preferendum, since every effort is made to remove extraneous stimuli, while a multitude of nonthermal stimuli interfere in a complex fashion with thermal responses in nature, making thermal distributions in the field less predictable. In extended laboratory tests, fish might similarly begin to respond to nonthermal factors, modifying the observed thermal distributions. Nutrition level may have an effect. Perhaps more significantly, social interactions in groups of fish seem to increase following the initial exploratory phase in a novel environment (W. W. Reynolds unpublished data), and such social interactions have been shown to affect thermal distributions and behavior (Bacon et al. 1967; Regal 1971; Beitinger and Magnuson 1975), especially in the case of subordinate individuals whose behavior is interfered with by socially dominant individuals. A great deal of further work is needed to more fully clarify all of the above considerations.

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Compilation of Temperature Preference Data[1]

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This report briefly summarizes current information from field and laboratory studies on temperature selection by fishes, with a tabulation of final temperature preferenda and upper and lower avoidance temperatures.

Key words: temperature, selection, preferendum, avoidance, preference, behavior, orientation

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On résume brièvement dans cet article les connaissances courantes acquises sur le terrain et en laboratoire sur le choix des températures par les poissons, avec tabulation des températures préférées finales et des températures d'évitement supérieures et inférieures.

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THIS paper summarizes information on temperatures selected by fishes in laboratory and field situations. Its primary purpose is to provide tabular data on three "endpoints" of temperature selection that have been found useful for setting temperature standards for water bodies and for describing and predicting the behavior of fishes near power station heated discharges (Table 1). These endpoints are the final preferendum and the upper and lower avoidance temperatures. A significant amount of additional data has been published since Coutant (1975) summarized reports through 1973.

Despite different study objectives and methods among the research reports summarized in Table 1, a pattern of temperature preference appears in the results for many species. Species specificity is clearly demonstrated with reasonable consistency among laboratory and field results. For some species, the tabular summary clarifies the need for research directed toward resolving contradictions. Discrepancies among results indicate where caution should be used in applying these data in impact assessments. Some tabulated values represent my own interpretation of the authors' data, which may differ from theirs. Certainly, the original papers should be fully understood before these data are used for power plant impact analyses or for other purposes.

Acknowledgments

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TABLE 1. Summary of available data on final preferendum and upper and lower avoidance temperatures of fish from field and laboratory studies. Numbers here may vary somewhat from the original author's interpretation. Sp = spring; W = winter; F = fall; Su = summer; YOY = young of the year.

Species	Size or age	Temp (°C) Upper avoidance	Final preferendum	Lower avoidance	Location	Reference
Alburnus alburnus (bleak)	Large	20			Lab	Alabaster and Downing 1966
Alosa chrysochloris (skipjack herring)	Large	29		22	Wabash R., Ind.	Gammon 1973
A. pseudoharengus (alewife)			18.8		Cayuga L., N.Y.	Galligan 1951
		22.0		8.0	L. Michigan	Wells 1968
	Adult (Sp)		21.3		Lab	Reutter and Herdendorf 1974
A. sapidissima (American shad)	Small	30			Connecticut R., Conn.	Marcy et al. 1972
Ambloplites rupestris (rock bass)			21.3		Wisconsin Lakes	Hile and Juday 1941
			20.7		S. Ontario streams	Ferguson 1958
			27–27.8[a]		L. Monona, Wis.	Neill 1971
			26.8–28.3[b]		L. Monona, Wis.	Neill 1971
	Small	29.0	26.2	25.5	Lab[a]	Neill 1971
	Small	29.5	28.8	26.0	Lab[b]	Neill 1971
	Adult (W)		21.6		Lab	Reutter and Herdendorf 1974
	Adult (Sp)		20.5		Lab	Reutter and Herdendorf 1974
	Adult (F)		22.8		Lab	Reutter and Herdendorf 1974
Anchoa mitchilli (bay anchovy)	All	33	24.5–32.5		Galveston Bay, Tex.	Gallaway and Strawn 1974
Aplodinotus grunniens (freshwater drum)	Large		22.2		Norris Res., Tenn.	Dendy 1948
	Large	30		22	Wabash R., Ind.	Gammon 1973
	Small		29.5–30.3[a]		L. Monona, Wis.	Neill 1971
	Small		27.5–29[b]		L. Monona, Wis.	Neill 1971
	YOY (Su)		31.3		Lab	Reutter and Herdendorf 1974
	Adult (Su)		26.5		Lab	Reutter and Herdendorf 1974
	Adult (F)		19.6		Lab	Reutter and Herdendorf 1974
Arius felis (sea catfish)	All	37			Galveston Bay, Tex.	Gallaway and Strawn 1974
Atherinops sp[c] (silverside species)		28	25.2	22	California coast	Doudoroff 1938
Brevoortia patronis (gulf menhaden)	All	30			Galveston Bay, Tex.	Gallaway and Strawn 1974
Campostoma anomalum (stoneroller)	All	23.8			New R., Va.	Sauffer et al. 1975[a]
			26.8		Lab	Stauffer et al. 1975[a]
	Adult	33	29	24	Lab	Cherry et al. 1975
Carassius auratus (goldfish)	Small		28.1		Lab	Fry 1947
	Small	33	30		Lab	Roy and Johansen 1970
	Adult (W)		24.2		Lab	Reutter and Herdendorf 1974
	Adult (Sp)		25.3		Lab	Reutter and Herdendorf 1974
	Adult (Su)		27.0		Lab	Reutter and Herdendorf 1974
	Adult (F)		24.0		Lab	Reutter and Herdendorf 1974
	Medium		27.9		Lab	Reynolds and Covert 1977
Carpoides carpio (river carpsucker)	Large	34.5		26	Wabash R., Ind.	Gammon 1973

TABLE 1 (*Continued*)

Species	Size or age	Temp (°C) Upper avoidance	Final preferendum	Lower avoidance	Location	Reference
C. cyprinus (quillback)	Large	34.5		27.0	Wabash R., Ind.	Gammon 1973
	Adult (F)		22.1		Lab	Reutter and Herdendorf 1974
Catostomus catostomus (longnose sucker)			11.6		Moosehead L., Me.	Cooper and Fuller 1945
C. commersoni (white sucker)			20.6		Wisconsin Lakes	Hile and Juday 1941
			18.3		Moosehead L., Me.	Cooper and Fuller 1945
	Large		18.9–21.1		Horsetooth Res., Colo.	Horak and Tanner 1964
	Adult (F)		22.4		Lab	Reutter and Herdendorf 1974
Chromis chromis (damselfish)		26	20	8	Lab	Cabanac and Jeddi 1971
Coregonus artedii (cisco)	Large	20	10		L. Nipissing, Ont.	Fry 1937
	Large		7.2		Cayuga L., N.Y.	Galligan 1951
C. clupeaformis (lake whitefish)			11.9		Moosehead L., Me.	Cooper and Fuller 1945
	Small		12.7		Lab	Ferguson 1958
	Larvae		17		South Bay, Lake Huron, Ontario	Reckahn 1970
	Larvae					
	12.9 mm		12–16		Lab	Hoagman 1974
	17.8 mm	17	13.5	12	Lab	Hoagman 1974
	23.1 mm	19	15.5	14.5	Lab	Hoagman 1974
C. hoyi (bloater)	Large	10		6	L. Michigan	Wells 1968
C. lavaretus (n.c.n.)	Larvae					
	(60 days)		11–15.4		Lab	Mantelman 1958
	(100 days)		8–12		Lab	Mantelman 1958
Cottus bairdii (mottled sculpin)			16.5		S. Ontario streams	Ferguson 1958
C. cognatus (slimy sculpin)	Large	6		4	L. Michigan	Wells 1968
Crenichthys baileyi (White River killifish)			29.5		White R., Nev.	Deacon and Bradley 1971
Cyprinodon macularius (desert pupfish)	Adults	36.5	35.5–36.5		Salton Sea, Calif.	Barlow 1958
	Young		40		Quitobaquito, Ariz.	Lowe and Heath 1969
Cyprinus carpio (carp)	Large	34.5		27	Wabash R., Ind.	Gammon 1973
	Large		29.3–31.9[a]		L. Monona, Wis.	Neill 1971
	Large		28.2–30.7[b]		L. Monona, Wis.	Neill 1971
	Young	35	32	28	Lab	Pitt et al. 1956
	Young	33.5	31.9	30	Lab[a]	Neill 1971
	Young	32.3	32	29.5	Lab[b]	Neill 1971
	Adult (Sp)		27.4		Lab	Reutter and Herdendorf 1974
	Adult (Su)		29.7		Lab	Reutter and Herdendorf 1974
	Adult	>31	29	24	Lab	Reynolds unpublished data
Cynoscion arenarius (sand seatrout)	All	40	29–32		Galveston Bay, Tex.	Gallaway and Strawn 1974
			35	30–35	Galveston Bay, Tex.	Copeland and Bechtel in Galloway and Strawn 1974
Dorosoma cepedianum (gizzard shad)	Large	30		23.5	Wabash R., Ind.	Gammon 1973
	Large		23.0		Norris Res., Tenn.	Dendy 1948
	Adult (F)		20.5		Lab	Reutter and Herdendorf 1974
Esox americanus vermiculatus (grass pickerel)	Small		26		Lab	Ferguson 1958
E. masquinongy (muskellunge)	Small		24		Lab	Ferguson 1958
E. niger (chain pickerel)	Medium	27	24	<20	Lab	Reynolds unpublished data
Gadus morhua morhua (Atlantic cod)	Large	8.9	1.4–2.0	0.6	Field	Tatyankin 1972
			1–3		Field	Tatyankin 1972
		8–10			Field	Tatyankin 1972
			1.5–2		Field	Tatyankin 1972
		15	9	1.0	Lab	Tatyankin 1972
	Small	16–17			Lab	Bohle 1974
Gambusia affinis (mosquitofish)			31		Field and Lab	Winkler 1973
	Adults	29.5	27		Lab	Bacon et al. 1967
	15–19 mm	32			Lab	Bacon et al. 1967
	<15 mm	35			Lab	Bacon et al. 1967
Gillichthys mirabilis (longjaw mudsucker)		23	22	9	Lab	deVlaming 1971
Girella nigricans (opaleye)	Transforming		28–31.2		California coast	Norris 1963
	55–60 mm		26–28.2		California coast	Norris 1963
		30	26		Lab	Duodoroff 1938
			26		Lab	Norris 1963
Heterodontus francisci (horn shark)	Young		24		Lab	Crawshaw and Hammel 1973

TABLE 1 (*Continued*)

Species	Size or age	Temp (°C) Upper avoidance	Final preferendum	Lower avoidance	Location	Reference
Hiodon alosoides (goldeye)	Large	28.5		22	Wabash R., Ind.	Gammon 1973
H. tergisus (mooneye)	Large	27		22	Wabash R., Ind.	Gammon 1973
Ictalurus natalis (yellow bullhead)	Adult (Su)		28.3		Lab	Reutter and Herdendorf 1974
I. nebulosus (brown bullhead)	Adult (W)		11.9		Lab	Reutter and Herdendorf 1974
	Adult (Sp)		23.5		Lab	Reutter and Herdendorf 1974
	Adult (Su)		24.9		Lab	Reutter and Herdendorf 1974
	Adult (F)		23.6		Lab	Reutter and Herdendorf 1974
	93–193 mm		27.3		Lab	Richards 1974
	Adult		29–31		Lab	Crawshaw 1975[b]
I. punctatus (channel catfish)	Large	32		26	Wabash R., Ind.	Gammon 1973
	Large	34			White R., Ind.	Proffitt 1969
	Adult (Su)		25.2		Lab	Reutter and Herdendorf 1974
	Adult (F)		25.3		Lab	Reutter and Herdendorf 1974
		35	30.5	23	Lab	Cherry et al. 1975
Ictiobus sp. (buffalo species)	Large	34.5		27	Wabash R., Ind.	Gammon 1973
Leiostomus xanthurus (spot)	All	37.5			Galveston Bay, Tex.	Gallaway and Strawn 1974
Lepisosteus osseus (longnose gar)	Large		30–31.8		L. Monona, Wis.	Neill 1971
	Large	34.5		29	Wabash R., Ind.	Gammon 1973
	YOY (Su)		25.3		Lab	Reutter and Herdendorf 1974
	Adult (Su)		33.1		Lab	Reutter and Herdendorf 1974
L. platostomus (shortnose gar)	Large	34.5		27	Wabash R., Ind.	Gammon 1973
Lepomis cyanellus (green sunfish)	< 74 mm	30	27.3	24	Lab	Jones and Irwin 1965
	Adult	33	30.6	23	Lab	Cherry et al. 1975
	Small	30.3	28.2	26.5	Lab	Beitinger et al. 1975
L. gibbosus (pumpkinseed)	Large		28.5–32[a]		L. Monona, Wis.	Neill 1971
	Large		27–29[b]		L. Monona, Wis.	Neill 1971
	Small		31.5		Lab	Ferguson 1958
	Adult (Sp)		24.2		Lab	Reutter and Herdendorf 1974
	Adult (Su)		27.7		Lab	Reutter and Herdendorf 1974
	Large	> 31	26	< 22	Lab	Reynolds unpublished data
L. macrochirus (bluegill)			29.4–31.3		L. Monona, Wis.	Neill 1971
	53–99 mm		28.8–31.2[a]		L. Monona, Wis.	Neill 1971
	53–99 mm		27–29[b]		L. Monona, Wis.	Neill 1971
	100–193 mm		29.6–32.6[a]		L. Monona, Wis.	Neill 1971
	100–193 mm		27.2–29[b]		L. Monona, Wis	Neill 1971
			32.3		Lab	Ferguson 1958
	Young	32.1	30.2	28.5	Lab[a]	Neill 1971
	Young	32.5	31.5	28.5	Lab[b]	Neill 1971
	Young	33.1	31.2	29.3	Lab	Beittinger 1974
	Adult (W)		27.4		Lab	Reutter and Herdendorf 1974
	Adult	35	32	26	Lab	Cherry et al. 1975
	45–110 mm	33.0	32.3	26	Lab	Reynolds and Casterlin 1976[a]
	120–155 mm		30.5		Lab	Reynolds et al. 1976[a]
Leuresthes sardina (gulf grunion)	25–160 days old	37	32	< 20	Lab	Reynolds and Thomson 1974[a]
L. tenuis (Calif. grunion)	Adult	34	25	< 20	Lab	Reynolds et al. 1977[a]
Lota lota lacustris (burbot)	Small		21.2		Lab	Crossman et al. 1953 *cited by Ferguson 1958*
L. lota maculosa (burbot)			11.4		Moosehead L., Me.	Cooper and Fuller 1945
Melanogrammus aeglefinus (haddock)			5–6		Field	Tatyankin 1972
Membras martinica (rough silverside)	All	33			Galveston Bay, Tex.	Gallaway and Strawn 1974
Menidia beryllina (tidewater silverside)	All	34			Galveston Bay, Tex.	Gallaway and Strawn 1974
M. menidia (Atlantic silverside)		32			Lab	Meldrim 1970
Micropogon undulatus (Atlantic croaker)	All	38			Galveston Bay, Tex.	Gallaway and Strawn 1974
Micropterus dolomieui (smallmouth bass)			21.3		Nebish L., Wis.	Hile and Juday 1941
			21.4		S. Ontario streams	Ferguson 1958
	Small		28.0		Lab	Ferguson 1958
	YOY (W)		18.0		Lab	Barans and Tubb 1976
	YOY (Sp)		19–24		Lab	Barans and Tubb 1973

TABLE 1 (*Continued*)

Species	Size or age	Temp (°C) Upper avoidance	Final preferendum	Lower avoidance	Location	Reference
	YOY (Su)		31		Lab	Barans and Tubb 1973
	YOY (F)		24–27		Lab	Barans and Tubb 1973
	YOY (F)		26.6		Lab	Reutter and Herdendorf 1974
	YOY	35	31.1	25	Lab	Reynolds and Casterlin 1976[a]
	Adult (W)		12–13		Lab	Barans and Tubb 1973
	Adult (Sp)		15–16		Lab	Barans and Tubb 1973
	Adult (Su)		30.0		Lab	Barans and Tubb 1973
	Adult (F)		21–23		Lab	Barans and Tubb 1973
		33	31.3	26	Lab	Cherry et al. 1975
M. punctulatus (spotted bass)	Large		24.4		Norris Res., Tenn.	Dendy 1948
	Large	27		22	Wabash R., Ind.	Gammon 1973
		34	32.5	27	Lab	Cherry et al. 1975
M. salmoides (largemouth bass)	Large		26.6–27.7		Norris Res., Tenn.	Dendy 1948
	Large	30	27–30		Par Pond. S.C.	Clugston 1973
	72–99 mm		29.3–30.9		L. Monona, Wis.	Neill 1971
	100–408 mm		29.3–32[a]		L. Monona, Wis.	Neill 1971
	100–408 mm		26.5–29.1[b]		L. Monona, Wis.	Neill 1971
	Adult	29	27	25.5	Small Tenn. Lakes	Coutant 1975
	Small		30–32		Lab	Ferguson 1958
	Small	30.7	29	27.5	Lab	Neill 1971
	Adult	30			Pond C., Savannah R. Plant, S.C.	Siler and Clugston 1975
	110–160 mm YOY		30.1		Lab	Reynolds et al. 1976[a]
	110–150 mm YOY	34	30	21	Lab	Reynolds and Casterlin 1976[a]
	50–460 g		30.2		Lab	Reynolds et al. 1976[b]
Moapa coriacea (Moapa dace)			29.5		White R., Nev.	Deacon and Bradley 1971
Morone americana (white perch)	Small	35	32		Lab	Meldrim and Gift 1971
M. chrysops (white bass)	Large	29			Wabash R., Ind.	Gammon 1973
	YOY (W)		10–13		Lab	Barans and Tubb 1973
	YOY (Sp)		16–18		Lab	Barans and Tubb 1973
	YOY (Su)		31.0		Lab	Barans and Tubb 1973
	YOY (F)		28.0		Lab	Barans and Tubb 1973
	YOY (Su)		27.8		Lab	Reutter and Herdendorf 1975
	Adult (W)		12–17		Lab	Barans and Tubb 1973
	Adult (Sp)		12–17		Lab	Barans and Tubb 1973
	Adult (Su)		28–30		Lab	Barans and Tubb 1973
	Adult (F)		16–17		Lab	Barans and Tubb 1973
M. mississippiensis (yellow bass)	Large		27.5–29.8[a]		L. Monona, Wis.	Neill 1971
	Large		27–28.7[b]		L. Monona, Wis.	Neill 1971
M. saxatilis	Small	34.4			Lab	Meldrim 1970, Meldrim and Gift 1971
(striped bass)	3 yr old	24	22	21	Small Tenn. Lakes	Coutant and Carroll unpublished data
Moxostoma sp. (redhorse species)	Large	26		22	Wabash R., Ind.	Gammon 1973
Myoxocephalus quadricornis (fourhorn sculpin)	Large	4.5 10		4	L. Michigan Field	Wells 1968 Westin 1970
Nocomis leptocephalus (bluehead chub)	Adult	17	15.0	10	Lab	Cherry et al. 1975
Notemigonus crysoleucas (golden shiner)	Adult (W)		16.8		Lab	Reutter and Herdendorf 1974
	Adult (Sp)		23.7		Lab	Reutter and Herdendorf 1974
	Adult (Su)		22.3		Lab	Reutter and Herdendorf 1974
	Adult (F)		21.0		Lab	Reutter and Herdendorf 1974
Notropis atherinoides (emerald shiner)	YOY (W)		10–12		Lab	Barans and Tubb 1973
	YOY (Sp)		13–15		Lab	Barans and Tubb 1973
	YOY (S)		22–23		Lab	Barans and Tubb 1973
	YOY (F)		13–14		Lab	Barans and Tubb 1973
	Adult (W)		5–6		Lab	Barans and Tubb 1973
	Adult (Sp)		16.0		Lab	Barans and Tubb 1973
	Adult (S)		22–24		Lab	Barans and Tubb 1973
	Adult (F)		15–16		Lab	Barans and Tubb 1973
	Adult (W)		9.3		Lab	Reutter and Herdendorf 1974
N. hudsonius (spottail shiner)	Large	>22		13	L. Michigan	Wells 1968
	Adult (W)		10.2		Lab	Reutter and Herdendorf 1974
	Adult (Sp)		14.3		Lab	Reutter and Herdendorf 1974
N. rubellus (rosyface shiner)	Adult	31	26.8	21	Lab	Cherry et al. 1975
N. spilopterus (spotfin shiner)	Adult	35	29.5	26	Lab	Cherry et al. 1975
Notorus flavus (stonecat)	Adult (W)		5.5		Lab	Reutter and Herdendorf 1974
	Adult (F)		25.1		Lab	Reutter and Herdendorf 1974

TABLE 1 (*Continued*)

Species	Size or age	Temp (°C) Upper avoidance	Final preferendum	Lower avoidance	Location	Reference
Oncorhynchus gorbuscha (pink salmon)	Small		11.7		Lab	Brett 1952
	Newly emerged		11.7–12.8		Lab	Hurley and Woodall 1968
	50 days		*9.3*		*Lab*	*Hurley and Woodall 1968*
O. keta (chum salmon)	Small		14.1		Lab	Brett 1952
O. kisutch (coho salmon)	Adult (Sp)		11.4		Lab	Reutter and Herdendorf 1974
	Adult		16.6		L. Michigan	Spigarelli 1975
O. nerka (sockeye salmon)			10.6–12.8		Cultus L., B.C.	Foerster 1937
					Horsetooth Res., Colo.	Horak and Tanner 1964
		21			Okanagan R., Wash.	Hajor and Mighel 1966
	Small		14.5		Lab	Brett 1952
O. tshawytscha (chinook salmon)	Small		11.7		Lab	Brett 1952
	Adult		17.3		L. Michigan	Spigarelli 1975
Osmerus mordax (rainbow smelt)			6.6–8.3		Cayuga L., N.Y.	Galligan 1951
		14		6	L. Michigan	Wells 1968
			12.8		L. Champlain, N.Y.	Greene 1930
Perca flavescens (yellow perch)	Small		12.2		Muskellunge L., Wis.	Hile and Juday 1941
	Large		20.2		Muskellunge L., Wis.	Hile and Juday 1941
			20.2		Silver L., Wis.	Hile and Juday 1941
			21.0		Nebish L., Wis.	Hile and Juday 1941
			20.8		Trout L., Wis.	Hile and Juday 1941
			19.7		L. Nipising, Ont.	Ferguson 1958
			21.2		L. Opeongo, Ont.	Ferguson 1958
			21.0		Costello L., Ont.	Ferguson 1958
				11	L. Michigan	Wells 1968
	Small		21.0		Lab	Ferguson 1958
	Small		24.2		Lab	Ferguson 1958
	Small	26.5	23.3	20.2	Lab[a]	Neill 1971
	Small	25	22.5	19.5	Lab[b]	Neill 1971
	Small		23.3		Lab	McCauley and Read 1973
	Adults		20.1		Lab	McCauley and Read 1973
	YOY (W)		10–13		Lab	Barans and Tubb 1973
	YOY (Sp)		18.0		Lab	Barans and Tubb 1973
	YOY (S)		25–27		Lab	Barans and Tubb 1973
	YOY (F)		28.0		Lab	Barans and Tubb 1973
	Adult (W)		7–12		Lab	Barans and Tubb 1973
	Adult (Sp)		13–16		Lab	Barans and Tubb 1973
	Adult (S)		27.0		Lab	Barans and Tubb 1973
	Adult (F)		22–25		Lab	Barans and Tubb 1973
	Adult (W)		14.1		Lab	Reutter and Herdendorf 1974
	Adult (S)		20.9		Lab	Reutter and Herdendorf 1974
	Adult (F)		19.9		Lab	Reutter and Herdendorf 1974
P. fluviatilis (Eurasian perch)		28.5			Polish lakes	Horoszewicz 1973
Percopsis omiscomaycus (trout-perch)	Adult	16		10	L. Michigan	Wells 1968
Pimephales promelas (fathead minnow)			28.5		Lab	Opuszynski 1971
	Adult	32	29	25	Lab	Cherry et al. 1975
	< 74 mm	28	23.4		Lab	Jones and Irwin 1965
P. notatus (bluntnose minnow)	Adult	31	29	21	Lab	Cherry et al. 1975
Pleuronectes platessa (plaice)	15–23 cm		16–17		Lab	Zahn 1963
Poecilia reticulata (guppy)	Adult male		28.2		Lab	Ruff and Zippel 1966
	Adult female		27.6		Lab	Ruff and Zippel 1966
	Adult		29		Lab	Ogilvie and Fryer 1971
Polydactylus octonemus (Atlantic threadfin)		33.5			Galveston Bay, Tex.	Gallaway and Strawn 1974
Pomoxis annularis (white crapple)	Large	27		22	Wabash R., Ind.	Gammon 1973
	Adult (W)		19.8		Lab	Reutter and Herdendorf 1974
	Adult (Sp)		18.3		Lab	Reutter and Herdendorf 1974
	Adult (F)		10.4		Lab	Reutter and Herdendorf 1974
P. nigromaculatus (black crapple)	Large		27.8–29.8[a]		L. Monona, Wis.	Neill 1971
	Large		27–28.2[b]		L. Monona, Wis.	Neill 1971
	Small	30		26.5	Lab[a]	Neill 1971
	Small	29.5		25.5	Lab[b]	Neill 1971
	Adult (W)		20.5		Lab	Reutter and Herdendorf 1974
	Adult (Sp)		21.0		Lab	Reutter and Herdendorf 1974
	Adult (Su)		21.7		Lab	Reutter and Herdendorf 1974
	Adult (F)		22.2		Lab	Reutter and Herdendorf 1974
	Medium	26	24	20	Lab	Reynolds unpublished data
Prosopium cylindraceum (round whitefish)			17.5		Moosehead L.	Cooper and Fuller 1945

TABLE 1 (*Concluded*)

Species	Size or age	Temp (°C) Upper avoidance	Final preferendum	Lower avoidance	Location	Reference
Pylodictus olivaris (flathead catfish)	Large	32		27	Wabash R., Ind.	Gammon 1973
Rhodeus sericeus (bitterling)	Adult		25		Lab	Zahn 1963
Rutilus rutilus (roach)			27		River Trent, UK	Alabaster and Downing 1966
	5–20 cm	25			Lab	Alabaster and Downing 1966
		28.5			Polish lakes	Horoszewicz 1973
Salmo gairdneri (rainbow trout)	Adult		18.9–21.1		Horsetooth Res., Colo.	Horak and Tanner 1964
	Red fingerlings		22		Lab	Javaid and Anderson 1967[a,b]
	Starved fingerlings		18		Lab	Javaid and Anderson 1967[a,b]
	Fingerlings	22–22	18–19	14	Lab	McCauley and Pond 1971
	Adult		13		Lab	Garside and Tait 1958
	Adult		16.5		L. Michigan	Spigarelli 1975
		19	18	13	Lab	Cherry et al. 1975
S. irideus (European rainbow trout)	Alevins	22	16–17	9	Lab	Mantelman 1958
	Fingerlings		14–15		Lab	Mantelman 1958
S. salar (Atlantic salmon)		14			Newfoundland lakes	Leggett and Power 1969
	Young		14		Lab	Fisher and Elson 1950
	Young		16		Lab	Mantelman 1958
	Underyearling		6–8		Lab	Ogilvie and Anderson 1965
	Young, fed		18		Lab	Javaid and Anderson 1967[a,b]
	Young, starved		20		Lab	Javaid and Anderson 1967[a,b]
S. salar sebago (landlocked Atl. salmon)			16.2		Moosehead L., Me.	Cooper and Fuller 1945
S. trutta (brown trout)			12		L. Oredon, France	James 1931
		20			Lab	Alabaster and Downing 1966
	Young		17.6		Lab	Ferguson 1958
	Adult		13.8		L. Michigan	Spigarelli 1975
Salvelinus fontinalis (brook trout)			19		Field	Creaser 1930
			20.3		Moosehead L., Me.	Cooper and Fuller 1945
			20.0		Redrock L. ,Ont.	Baldwin 1948
		20			Field studies	Smith and Saunders 1958
	Adult		15.7		S. Ontario streams	Ferguson 1958
	Adult		14.8		L. Michigan	Spigarelli 1975
	Small		16		Lab	Graham 1949
	Small, fall	20	16		Lab	Sullivan and Fisher 1953, 1954
	Small, winter		8–12		Lab	Sullivan and Fisher 1953, 1954
	Small, fed		18		Lab	Javaid and Anderson 1967[a,b]
		20	18	14	Lab	Cherry et al. 1975
	Small, starved		16		Lab	Javaid and Anderson 1967[a,b]
	Young		16		Lab	Peterson 1973
S. namaycush (lake trout)			14		White L., Ont.	Kennedy 1941
		13			Moosehead L., Me.	Cooper and Fuller 1945
		11			Lac La Ronge, NWT	Rawson 1956
			10		Louisa and Redrock L., Ont.	Martin 1952
			15.5		Cayuga L., N.Y.	Galligan 1951 (in Ferguson 1958)
	Young	15	11.7		Lab	McCauley and Tait 1970
	Young	14	11.5		Lab	Goddard et al. 1974
	Adult		11.8		L. Michigan	Spigarelli 1975
S. fontinalis × *S. namaycush* (splake)	Young		13.1 12.0		Jack and Sproule L., Ont. Lab	Ferguson 1958 Ferguson 1958
Scardinius erythrophthalmus (rudd)		28.5			Polish lakes	Horoszewicz 1973
Scorpaena scorfa (scorpionfish)		26	20	8	Lab	Cabanac and Jeddi 1971
Stizostedion canadense (sauger)	Large		19.2		Norris Res., Tenn.	Dendy 1948
	Large	28		22	Wabash R., Ind.	Gammon 1973
S. v. vitreum (walleye)	Large		20.6		Trout Lake, Wis.	Hile and Juday 1941
	Large		23.2		Norris Res., Tenn.	Dendy 1948
Thymallus thymallus (grayling)		18			Lab	Alabaster and Downing 1966
Tilapia mossambica (Mozambique mouthbrooder)		33.5	28.5		Lab	Badenhuizen 1967
T. nilotica			28–29.5		Lab	Beamish 1970
Tinca tinca (tench)		26			Lab	Alabaster and Downing 1956

[a]Day; [b]Night; [c]May be other genera as well. See Reynolds et al. 1977a.

Application of Temperature Preference Studies to Environmental Impact Assessment

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GIFT, J. J. 1977. Application of temperature preference studies to environmental impact assessment. J. Fish. Res. Board Can. 34: 746–749.

Increasing use of laboratory and field temperature preference and avoidance data in power plant siting and environmental impact assessment studies has created a need for evaluation and standardization of methodologies. Relevant considerations and applications are discussed.

Key words: temperature, preference, avoidance, power plant siting, environmental impact assessment

GIFT, J. J. 1977. Application of temperature preference studies to environmental impact assessment. J. Fish. Res. Board Can. 34: 746–749.

L'usage accru de données sur la préférence et l'évitement des températures en laboratoire et sur le terrain dans la détermination des sites de centrales d'énergie et dans l'évaluation des répercussions sur l'environnement crée un besoin d'évaluer et d'uniformiser les méthodes. Nous analysons dans cet article certaines considérations et applications pertinentes.

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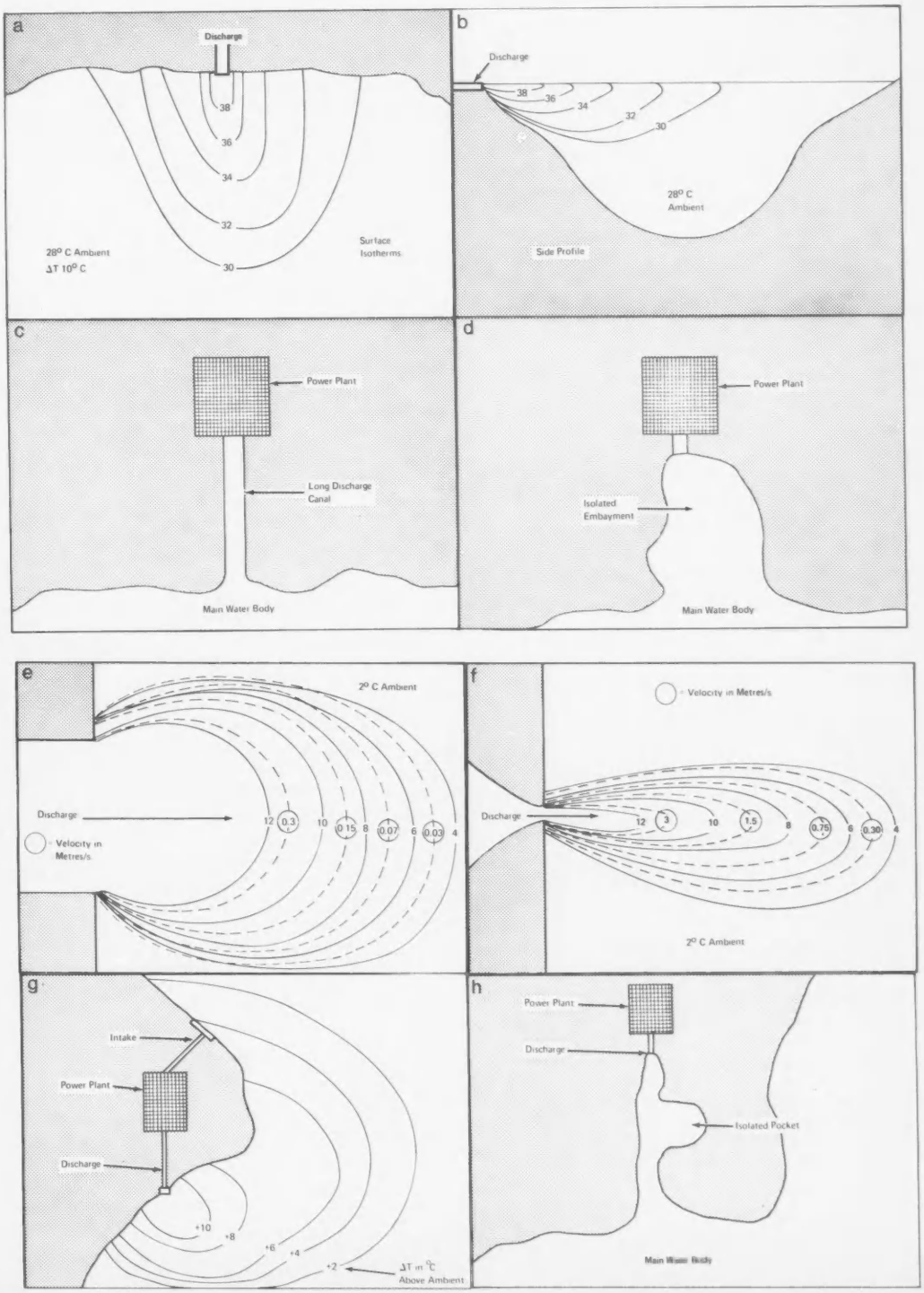
WITH recognition of increased demand for electrical power production in the late 1950s and early 1960s came predictions of massive fish mortalities due to lethal temperatures of thermal discharges from generating stations. However, few such large fish kills directly attributable to lethal temperatures have actually occurred, apparently because of the ability of fishes to sense and avoid lethal temperatures. In the past 15 years or so, a number of applied studies of the behavioral responses of fishes and macroinvertebrates to temperature gradients have been directed toward evaluation of the environmental impact of thermal discharges, many of them addressing site-specific problems with unique apparatus and methodologies (e.g. Alabaster 1963; Alabaster and Downing 1966; Moss 1970; Gift 1970; Meldrim and Gift 1971; Barans and Tubb 1973; Otto 1973; Richards 1974; Meldrim et al. 1974; Neill and Magnuson 1974; Cherry et al. 1975; Stauffer et al. 1975a, 1975b; Coutant 1975; Kelso 1976).

Growing recognition of the importance of behavioral studies in thermal discharge impact evaluations can be seen when successive drafts of the U.S. Environmental Protection Agency's 316(a) *Technical Guidance Manual* are reviewed. The U.S. Federal Water Pollution Control Act, Section 316(a), requires that thermal effluents must comply with strict limitations, unless the owner or operator of the discharge can demonstrate that the effluent limitations are more stringent than necessary to assure the protection and propagation of a balanced indigenous community of shellfish, fish, and wildlife in or upon the receiving water body. Section 316(a), Type II Demonstration guidelines require a list of thermal effects information for selected representative important species. An early version of the guidelines (U.S. Environmental Protection Agency 1974a) included no direct requirement for behavioral information. A later version (U.S. Environmental Protection Agency 1974b) requested data on upper lethal temperatures during reproductive and summer seasons, optimum temperatures for growth, minimum avoidance temperatures (a confusing term), and maximum temperatures for development. This direct requirement for incorporation of behavioral data into the 316(a) evaluation helped focus the need for

FIG. 1. a, Surface view of hypothetical summer isotherms; b, Depth profile of hypothetical summer isotherms; c, Power plant with a long discharge canal; d, Power plant discharging into an isolated bay where little mixing takes place; e, High-velocity discharge where high ΔT's are unavailable to fish due to high discharge velocities; f, Low-velocity discharge where high ΔT's are available to fish; g, Power plant site where a portion of the thermal discharge is recycled into the intake, increasing probability of impingement; h, Power plant site with an isolated pocket which can form a thermal "trap."


a
Discharge
38
36
34
32
30
28° C Ambient
ΔT 10° C
Surface Isotherms
b
Discharge
38
36
34
32
30
28° C Ambient
Side Profile
c
Power Plant
Long Discharge Canal
Main Water Body
d
Power Plant
Isolated Embayment
Main Water Body
e
2° C Ambient
Discharge
Velocity in Metres/s
12
0.3
10
0.15
8
0.07
6
0.03
4
f
Velocity in Metres/s
Discharge
12
3
10
1.5
8
0.75
6
0.30
4
2° C Ambient
g
Intake
Power Plant
Discharge
+10
+8
+6
+4
+2
ΔT in °C
Above Ambient
h
Power Plant
Discharge
Isolated Pocket
Main Water Body

accurate and reproducible temperature preference and avoidance studies.

The requirement for data on thermal growth optima also suggests a possible indirect use of behavioral information. Laboratory growth studies are time consuming, and few data are available for important forage species, as only important sport and commercial species have been studied. If final temperature preferenda can be related reliably to growth and other physiological optima, much time and expense could be saved in projecting growth data from the behavioral responses.

The site-specific nature of many applied studies and their use of unique apparatus and methodologies raise several pertinent questions. Is information from one site applicable to another? Are there geographic or populational differences in the thermal responses of a species? Only through some recognized standardization of methodology can these questions be answered and the degree of reproducibility established (cf. Reynolds and Casterlin 1976a).

In evaluating behavioral responses to potentially stressful chemical or physical conditions, we must also consider how a temperature response may interact with responses to other environmental factors. For example, J. W. Meldrim et al. (unpublished data) found that golden shiner (*Notemigonus crysoleucas*) avoided levels of approximately 110–115% gas supersaturation. However, as soon as a temperature increase of 5–10°C was associated with the supersaturated conditions, golden shiner exhibited a preference for the warmer supersaturated water. Such a situation can occur in discharge canals, where temperature attracts fish into supersaturated concentrations of dissolved gases which may cause gas-bubble disease. Kleerekoper et al. (1973) found an interaction of responses by goldfish (*Carassius auratus*) to temperature and copper ions. Reynolds et al. (1977) studied interacting responses to simultaneous gradients of temperature and light intensity in California grunion (*Leuresthes tenuis*), where the temperature response predominated.

Combining field with laboratory studies is highly desirable for accurate environmental impact assessments. We need data on the temporal and spatial distributions of fishes in the receiving water body as a whole, as well as in the vicinity of the thermal plume. Some important questions are: (1) What species are found in the discharge area year-round? (2) What species use the discharge area as a spawning, nursery, or foraging habitat? (3) What species migrate or pass through the discharge area? A fish can respond to a thermal discharge only if it comes into contact with it. Otherwise, it is not necessarily influenced by the heated water, although there may be indirect ecological effects. Field studies should help to indicate which species are likely to come into contact with a thermal plume.

Assuming that adequate information has been assembled on species populations, life histories of important species, and morphological and hydrological characteristics of the proposed site or sites, the next step is to assemble information on behavioral responses of the important species to temperature. This may require new studies, or may already be available in the literature. How can this information be used?

A first step is to project the thermal plume configuration during various seasons of the year, as in the hypothetical summer example of Fig. 1a and 1b. With this seasonal information it is possible to calculate the percentage of a water body affected by each isotherm under different hydrothermal conditions. Field and laboratory behavioral studies can predict what species are likely to be attracted or repelled by the heated area; what spawning, nursery, or habitat areas might be impaired or lost to various populations; or whether migratory routes might be blocked. Acoustic or radio temperature telemetry and tracking studies (cf. Coutant 1975; Kelso 1976) may provide valuable information on these matters.

Combining biological survey information, hydrothermal maps, and behavioral data can help to project the potential impact of a power plant effluent on fish species and can help in choosing a plant site with minimal detrimental impact on the biota. The danger of cold shock is a particularly important consideration in temperate climates, where many species are attracted to warm water during colder seasons. If fish remain in the discharge, they will become acclimated to the warm temperatures and thereby become susceptible to cold shock if the plant is shut down abruptly during cold weather. Discharge into a long canal (Fig. 1c) or into an isolated embayment (Fig. 1d) presents a high potential for cold shock, as fish are likely to be attracted to and to remain in such restricted warm areas which have limited mixing with ambient waters. To minimize cold shock danger a discharge configuration should have a minimal area of high ΔT and produce rapid mixing with ambient waters by using a high-velocity discharge (Fig. 1e) where the highest ΔT's are associated with discharge velocities high enough to prevent fishes from maintaining themselves within the higher temperatures long enough to become acclimated.

By contrast, a low-velocity discharge (Fig. 1f) represents a high risk of cold shock, with large heated areas in which fish can remain for long periods and become warm acclimated. Where a portion of a thermal discharge is recirculated into the intake (Fig. 1g), increased impingement problems can also result due to thermal attraction to the intake area. An isolated thermal pocket (Fig. 1h) can become a trap for organisms avoiding high discharge temperatures in summer, as organisms can follow a decreasing thermal gradient into this pocket rather than out into the open water. A further increase in temperature above lethal limits could kill all the entrapped organisms in the pocket. This type of configuration should always be avoided if possible.

These examples illustrate some of the ways in which behavioral data can be used to help predict the environmental impact of a thermal discharge. The ability of fish to perceive and respond to temperature gradients and the quantification of these temperature responses provide important information that should be included in power plant siting studies, preoperational evaluations, and general impact analyses.

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Laboratory Methods for Determining Temperature Preference

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MCCAULEY, R. W. 1977. Laboratory methods for determining temperature preference. J. Fish. Res. Board Can. 34: 749–752.

Different types of temperature-gradient devices used in the laboratory to determine temperature preferences of fish are classified and reviewed. The type of device used seems to have less effect on experimental results than do other variables such as age, size, season, physiological state, or social interactions.

Key words: preferred temperature, thermal gradients, thermoregulation, behavioral, gradient devices, laboratory techniques

MCCAULEY, R. W. 1977. Laboratory methods for determining temperature preference. J. Fish. Res. Board Can. 34: 749–752.

L'auteur classifie et passe en revue différents types d'appareils de gradient de température utilisés en laboratoire pour déterminer les préférences thermiques des poissons. Le type d'appareil utilisé semble avoir moins d'effet sur les résultats expérimentaux que certaines autres variables telles que l'âge, la taille, la saison, la condition physiologique ou les interactions sociales.

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PREDICTIVE information on the responses of fishes to natural temperature gradients can be economically obtained by studying responses to laboratory gradients, in which extraneous factors can be rigidly controlled, although results must be applied cautiously to more complex field situations. Concern for the effects of heated effluents on fishes in natural waters has stimulated interest in the directive effects of temperature in many laboratories. This paper briefly reviews the various types of gradient devices and experimental methods that have been used.

Since Fry's (1958) comprehensive review and classification of laboratory gradients, several important new techniques and devices have been developed, including electronic shuttleboxes (Frank and Meyer 1971, 1974; Neill et al. 1972; Reynolds et al. 1976b; Reynolds 1977a), which develop a temporal gradient through the operant behavior of the fish, body-core temperature tele-

metry (McCauley and Huggins 1976; Reynolds et al. 1976b), and calorimetry to measure average body temperature (Crawshaw 1976; Müller and Fry 1976).

Two-Chambered Devices

This classical gradient design (Shelford and Allee 1913) employs two streams of water differing in temperature that enter at opposite ends and flow toward a central drain, presenting a fish with a choice of two temperatures. Modifications of this design have recently been used to study avoidance temperatures (Otto 1973; Meldrim and Gift 1971; Meldrim et al. 1974). In theory the temperature preference of a fish can be measured by determining its choices among a series of paired temperatures, and this principle has been used in the development of electronic shuttlebox devices (see below) in which the fish itself determines the choices.

Radial or "Rosette" Devices

In this design (Fig. 1) a central introduction chamber is surrounded by a number of peripheral chambers each containing water of a different temperature. Water flows from the peripheral chambers into a central drain. The design shown in Fig. 1 (after K. McLean and F. E. J. Fry, University of Toronto) has not previously been published. A similar device has been used by Kleerekoper et al. (1973) to study interacting responses of goldfish (*Carassius auratus*) to temperature and copper ions.

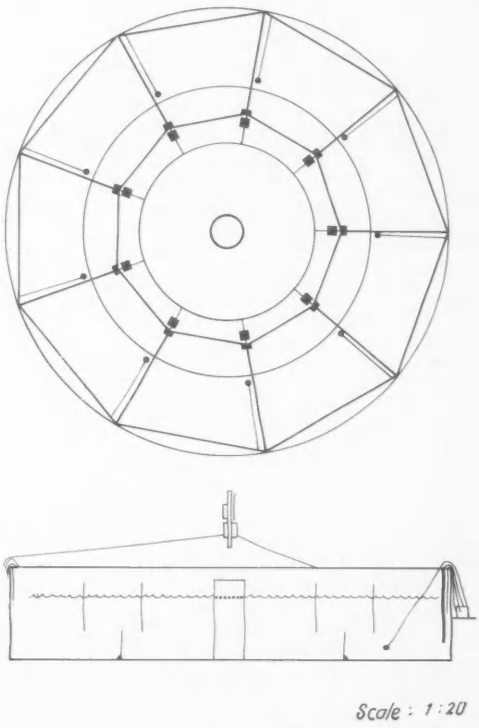

FIG. 1. A schematic diagram of a "rosette" gradient device (after K. McLean and F. E. J. Fry, University of Toronto).

Horizontal Linear Gradient Tanks

These come in two varieties. One is a long continuous trough in which water flows from one end to the other while being gradually heated or cooled (the former system can produce gas supersaturation). This gives a fairly smooth gradient, but presents a potential problem of fish orienting to the water flow (pseudorheotaxis). There are also difficulties in maintaining laminar flow and in preventing horizontal temperature lamination, usually necessitating shallow water depths that can only be used with small fish (Sullivan and Fisher 1954; Javaid and Anderson 1967a; Roy and Johansen 1970). The alternative solution is a deeper tank divided into a series of discrete chambers, in which lamination is prevented by vigorous aeration in each chamber (this also maintains oxygen levels while preventing supersaturation). The partitions between chambers reduce mixing, so the gradient consists of a stepwise series of discrete choices. No water flow is necessary. The apertures in the partitions must be of a size, shape, and depth suitable for the species (some species may have difficulty in negotiating the baffles and apertures). Variations of this chambered-trough design have been used often (e.g. Barans and Tubb 1973; Reynolds 1973; Reutter and Herdendorf 1974; Reynolds and Thomson 1974a; Reynolds et al. 1977).

Crossed-Gradient Devices

A two-axes multichamber crossed-gradient device has been described (Reynolds 1973; Reynolds and Thomson 1974a) which can be used to study simultaneous responses to two different gradients (e.g. temperature and light intensity) along two axes. Responses to the two gradients can thus be independent of one another. The particular device used, called the Selectatron, employed 16 chambers in a 4×4 configuration. An electronic shuttlebox version of this (see below) using four chambers in a 2×2 configura-

tion (the Ichthyotron) has also been described (Reynolds and Casterlin 1976a; Reynolds 1977a).

Transverse Gradients

In this type of device (cf. Fry 1958) a fish has a choice of several converging flows of water. This may be in the form of a simple *Y*-junction (e.g., Chidester 1921; Collins 1952) or may present more than two choices, as in Höglund's (1951) "Fluvarium."

Vertical Gradients

Vertical gradient tanks make use of vertical density stratification of water, such as occurs in lakes. This can involve a sharp thermocline which presents oxygenation problems in the "hypolimnion" or can employ a slow vertical flow of water which is either heated as it rises (with a danger of gas supersaturation and gas-bubble disease) or cooled as it descends. Several such devices have been used (Pearson 1952; Pitt et al. 1956; Ferguson 1958; Fry 1958; Garside and Tait 1958; McCauley and Tait 1970). This type of device presents the possibility that fish may respond to light intensity or hydrostatic pressure (water depth) as well as to temperature.

Electronic Shuttleboxes

Rozin and Mayer (1961) used operant-conditioning techniques that permitted goldfish to control water temperature by pressing against a lever. Van Sommers (1962) improved on this in allowing fish to control oxygen content of the water by interrupting a photocell light beam. Frank and Meyer (1971, 1974) used a partitioned shuttlebox monitored by a single photocell to study activity and thermoregulatory behavior of fish. Neill et al. (1972) described a two-chamber electronic shuttlebox device with a pair of photocells monitoring the movements of a fish and permitting it to control the temperatures in the two chambers, and thereby to control its own body temperature by shuttling back and forth. Other workers (e.g. Beitinger 1974; Reynolds et al. 1976b) have used variations of this design, including a four-chamber, two-axes design (Reynolds and Casterlin 1976a; Reynolds 1977a) for studying simultaneous responses to two variables. A small difference between the temperatures of the two chambers (about 1–2 deg C) presents an instantaneous spatial gradient to guide the movements of the fish and thereby minimizes the necessity of learning, in addition to the potential temporal gradient generated by the movements of the fish through the photocells. However, even with no temperature difference between chambers, fish will learn to control their body temperature in a purely temporal gradient (Neill et al. 1972). The body temperature of a fish averaged over time in a self-controlled shuttlebox does not differ significantly from the mean occupied temperature over that time period (Reynolds et al. 1976b). This type of device is ideal for allowing fish to gravitate to the final preferendum or for studying circadian rhythm effects (Reynolds and Casterlin 1976a), because of automatic data acquisition. Only a single fish can be tested at a time, however.

Toroidal Temporal Gradients

For constantly swimming pelagic fish like the tuna, a shuttlebox configuration is unsuitable. This has prompted development of a toroidal-shaped device (Neill et al. 1972) that permits a fish to control water temperature by its direction of swimming, forming a purely temporal gradient. Swimming in one direction constantly increases the temperature, while swimming in the other direction decreases temperature. A memory process in the fish permits association of swimming direction with either increase or decrease of temperature, which seems to be readily learned. Photocells around the periphery of the torus monitor speed and direction of swimming and control water temperature.

Body-Temperature Telemetry

Crawshaw (1975a) used implanted thermistors with trailing wires to monitor body temperatures of fishes moving in a gradient. More recently, miniature temperature telemetry transmitters small enough to be swallowed by a fish have been used to monitor body core temperatures of fishes, without necessity of trailing wires or surgery, in conventional linear laboratory gradients (McCauley and Huggins 1976) and in an electronic shuttlebox (Reynolds et al. 1976b). Because of thermal equilibration lag (dependent on the size of the fish), deep body temperatures fluctuate less than do occupied (ambient) temperatures in fishes moving in a gradient or shuttling back and forth. Crawshaw (1976), McCauley and Huggins (1976), and Reynolds (1977b) have examined thermal equilibration rates of fishes, which are affected by cardiovascular and respiratory variables.

Calorimetry

Calorimetric techniques have been used to measure the overall mean body temperatures of fishes following rapid temperature changes (Craw-

shaw 1976; Müller and Fry 1976), and as a means of estimating preferenda.

Comparison of Results from Different Methods

Comparison of results from different studies is often confounded by different techniques, apparatus, and statistical measures used by different investigators, but in spite of this difficulty there has been fairly good general agreement (cf. Zahn 1962; Fry 1967; Reynolds and Casterlin 1976a), with differences in final preferendum values for a particular species ranging typically within ±4 deg C when age of the fish and season are taken into account. A noteworthy case history is that of the rainbow trout (*Salmo gairdneri*).

In a vertical gradient, 1–2-year-old trout had a final preferendum of 13.6°C (Garside and Tait 1958). However, young fry of this species exhibited a final preferendum about 5 deg C higher in a horizontal gradient (Javaid and Anderson 1967a). McCauley and Pond (1971) compared the responses of young fry in vertical and horizontal gradients and found the same final preferendum of 18°C in both devices, indicating that the differing results of the previous studies could be attributed to age differences rather than to the apparatus used. Two more recent studies further support the presence of an ontogenetic decrease in final preferendum of *S. gairdneri*: 15-month-old trout showed a final preferendum of 11.6°C in an electronic shuttlebox (McCauley et al. 1977), while 13-month-old trout had a final preferendum of 16°C in a rosette device (W. J. Christie, Glenna Fisheries Research Station, Picton, Ontario, personal communication).

I conclude that while there may be some influence of apparatus and methodology on preferred-temperature values, variability due to effects of age, size, and probably also season, physiological condition, and social factors are likely greater than experimental artifacts.

Acknowledgments

This work was supported in part by grants from the U.S. Environmental Protection Agency and the Ontario Hydro. I thank Dr W. W. Reynolds for helpful suggestions during the preparation of this review and F. Paul Richards for his organizational efforts and encouragement.

Panel Discussion

The hour-long discussion following presentation of the five papers was taped. The transcripts have been severely edited and the comments paraphrased for brevity. The principal symposium participants are identified throughout by their initials: FPR = F. Paul Richards; LIC = Larry I. Crawshaw; WWR = William W. Reynolds; RWM = Robert W. McCauley; JJG = James J. Gift; CCC = Charles C. Coutant.

FPR — Should fish be laboratory acclimated, or field-acclimatized (cf. Norris 1963)?

RWM — Both ways, if possible, for comparison.

LIC — Extremely precise acclimation (± 0.1–0.5°C) may amount to a stimulus-deprivation situation.

WWR — My grunion (Reynolds and Thomson 1974a) showed the same final preferendum whether acclimated at fixed or at cycled temperatures.

CCC — The thermal history of fish collected from the field is unknown, so some laboratory acclimation is usually necessary.

JJG — To study seasonal effects, fish should be collected at different seasons.

RWM — It is often difficult to laboratory-acclimate sensitive species like smelt and alewives.

J. R. Stauffer — Preference-acclimation curves can be read in reverse (once we have baseline data) to determine field acclimatization state.

R. L. Kendall — We need a precise operational definition of acclimation.

RWM — Does "preference acclimation" proceed at the same rate as "tolerance acclimation?"

WWR — There is a phenomenon called "low thermal responsiveness" (Beitinger and Magnuson 1976), in which fish gravitate too rapidly to the final preferendum. These cold-acclimated fish select lethal warm temperatures to which they are not acclimated. In this case, the behavioral acclimation mechanism apparently responds faster than the physiological one.

FPR—Let's discuss Fry's (1947) bipartite definition of the final preferendum.

WWR — Fry defined the final preferendum as (1) that temperature at which acclimation and preference are equal and also (2) that temperature toward which a fish will finally gravitate regardless of its previous thermal history. This has led to two different methodologies for determining the final preferendum. Some workers merely allow the fish to gravitate to the final preferendum; when a stable preferendum is reached, usually over a 2–3-day period, this is inferred to be the final preferendum. The other basic method is to determine short-term prefer-

enda (over a 2-hour period or less) of fish acclimated at a series of different temperatures, to determine the relationship between acute preference and acclimation temperatures. The relationship is often nonlinear (cf. Zahn 1962; Fry 1967), so graphical methods are preferable to linear regressions (e.g. Hill et al. 1975) to determine where the preference and acclimation lines cross. More comparisons of results between the "gravitation" and "acute" methods may be needed to substantiate the degree of agreement between them, although available data indicate a fairly good correspondence (cf. Badenhuizen 1967).

CCC — I don't know of any substantially conflicting data between the two methods.

WWR — It depends on what degree of precision you're looking for. There is good general agreement among studies within a few degrees or so. We cannot now get reproducibility down to tenths of a degree Celsius.

CCC — Linear regressions of acute preferenda on acclimation temperature are inappropriate, since the relationship is usually nonlinear. Sometimes we lose the biology in statistics. In tabulating results from the literature, I threw out linear regressions and "eyeballed" the final preferendum from the data points.

FPR — What of water quality considerations?

JJG — Site-specific studies are often desirable but not always practical. Variables such as salinity and pH seem to have a minor effect on thermal preference. More important is the possibility of gas supersaturation when cold water is heated in a gradient. Measures must be taken to prevent this; one way is to cool and aerate warm water in establishing the gradient.

WWR — Gulf grunion (Reynolds and Thomson 1974a) avoided unnaturally high oxygen concentrations (about 20 mg/ℓ) corresponding to supersaturated conditions, as well as low oxygen levels (less than 5 mg/ℓ).

FPR — Are there seasonal effects on the final preferendum?

JJG — We're still not sure whether or to what extent they may exist, but if they do they would be important in impact assessment.

RWM — There is evidence for seasonal effects in some species, e.g. alewives.

WWR — Any such difference may be largely attributed to photoperiod, which in any experiment must be controlled and/or specified. There could also be an endogenous circennial rhythm involved, which would be merely entrained and not controlled by photoperiod, but evidence for this is still slim. Sorting this out will require careful long-term studies.

CCC — I don't think any such seasonal effects on final preferendum are of sufficient magnitude to require consideration in impact assessment.

FPR — What of circadian or diel thermoperiodism?

WWR — The modal preferendum is the best statistic to show this; it changes more than other parameters between night and day as skewness changes (Reynolds and Casterlin 1976a). Standardization could involve testing at one specified time of day, or pooling data for 24-hour periods (cf. Reynolds et al. 1976a) to "iron out" rhythm effects.

W. Lifton — In the field, there are also responses to changing light conditions and movements of prey organisms. How can we tell if a response is only to temperature?

WWR — This is exactly the reason for laboratory experiments: we can control extraneous variables to ensure that responses are to temperature only.

A. B. Stasko — I'm still worried about responses to nonthermal conditions in field telemetry experiments.

R. L. Kendall — Clupeids in Lake Tanganyika show diel vertical migrations through a thermal gradient equal to the entire seasonal cycle of surface temperatures.

FPR — Should fish be tested individually or in groups?

CCC — Schooling species often behave abnormally when isolated.

A. B. Stasko — Sullivan found that a group of blinded trout behaved most predictably!

WWR — A group of fish when placed in a gradient initially show exploratory behavior, ignoring each other, for the first few days; after that they may start to set up territories and/or dominance hierarchies if they are not true schooling species. Schooling fishes will tend to move as a unit if they have enough room.

JJG — It helps to know the natural history and social structure of the species under study, and test them accordingly.

J. R. Stauffer — *Notropis* spp. tested both individually and in groups gave similar results, but the isolated individuals were much more excitable and took longer to "settle down."

FPR — What of size/age effects?

CCC — There are some age differences. Fishermen are more concerned with adults. Young fish are more important for recruitment. There is some intraspecific size segregation corresponding to different thermal preferences, helping to reduce cannibalism.

WWR — Larval gulf grunion don't develop a temperature response until they're postlarvae 3 wk old. This isn't due to limited locomotor ability, since even newly hatched prolarvae respond

strongly to light, both vertically and horizontally (cf. Reynolds and Thomson 1974a). Beitinger and Magnuson (1975), however, found no age or size differences in preferenda or juvenile and adult bluegills, and I found that juvenile and adult largemouth bass prefer about the same temperatures.

FPR — What statistics should be used to characterize temperature responses?

WWR — Pitt et al. (1956) pointed out that the mode of the preferred-temperature distribution best corresponds to the definition of preferred temperature as that temperature occupied most frequently. The mode also best reflects day–night thermoperiodism.

W. C. Leggett — Is there a danger of overstandardization of methodology stifling creativity?

RWM — I doubt it; we all have our own favorite gadgets and methods.

WWR — There may be greater danger of a lack of consensus about how best to proceed.

LIC — We must often tailor the apparatus and methodology to the particular species under study.

JJG — Our main consideration here is really to identify the important factors that must be considered in designing an experiment.

CCC — The application of temperature preference data to environmental impact assessments (and 316a demonstrations) has given them a new importance. A diversity of methodological approaches and interpretations appears rather haphazard and disconcerting to the lawyers and politicians. This is one good reason for seeking some degree of consensus on methodology and interpretation. Diversity of approach can often be less appropriate in applied science than in basic science where creativity is more desirable.

Recommendations

The somewhat tentative nature of these procedural suggestions illustrates the many remaining gaps in knowledge requiring further research. The following points should be considered in planning temperature preference experiments:

Acclimation — There is little specific information on the rate of thermal preference acclimation, but it seems to proceed rapidly in the "upward" direction during gravitation to the final preferendum and may even exceed the rate of physiological acclimation, resulting in heat death (LTR; cf. Beitinger and Magnuson 1976). Conservative suggested holding times prior to acute experiments are 1 week for upward acclimation and 1 month for downward acclimation. Acclimation is less critical for "gravitation" experiments, which in any case must be of sufficient duration to achieve a stable preferendum. Reynolds and Thomson (1974a) found no difference in final preferenda of fish previously held at fixed or at cycled temperatures. Some sensitive species may be difficult to either acclimate or test for extended periods due to excessive mortality.

Final preferendum determination — Fish can be allowed to gravitate to a stable final preferendum. If a series of acute experiments (2 hours or less) with differently acclimated groups of fish are used, graphical estimation is preferable to linear regression, since the relationship between preferred and acclimation temperatures is generally nonlinear.

Seasonal effects — Fish should be brought directly from the field to test for seasonal effects; otherwise they must be kept at ambient seasonal temperatures and photoperiods until testing (unless free-running circennial rhythms are the subject of investigation).

Circadian (diel) rhythms — Thermoperiodism occurs in at least some fishes just as it does in endotherms. Where such rhythmic effects occur, fish should either be tested at a standard and specified time of day, or else data can be pooled for 24-hour periods to "smooth out" cyclic variations. The modal preferendum best shows such rhythms. Electronic shuttleboxes with automatic data acquisition are most convenient for studying rhythms (cf. Reynolds and Casterlin 1976a).

Water quality — The danger of gas supersaturation and gas-bubble disease must be avoided when water is heated or released from pressure. Vigorous aeration, or cooling of warm water, is a suggested countermeasure. Toxic substances such as chlorine or pesticide residues, as well as accumulations of toxic metabolites such as ammonia, should be filtered from water in holding or testing tanks. Overcrowding of fish produces disease outbreaks, which can alter preferenda.

Group vs. individual testing — Only individual fish can be tested in electronic shuttleboxes. Territorial species should be tested singly. For schooling species which do not behave normally when

isolated, groups should be tested and statistically treated as a unit. Social interactions of a species must always be considered in experimental design.

Size/Age of test specimens — The size or age of test animals should always be specified in reporting results, in view of possible ontogenetic preference changes.

Feeding schedule — Fish should be fed before, but usually not during tests, to avoid interfering with temperature responses. In extended tests, some feeding may be necessary; changes in behavior should be noted. A randomized (temporally and spatially) feeding schedule will prevent learning effects that could cause experimental artifacts.

Shape and size of test tanks — Tanks should be large enough to permit normal behavior. Schooling species will not school in crowded quarters, and may require rounded corners as well. Some species may need shelter. Apertures must be designed so as to be readily negotiable by the test species (physically and psychologically).

Control for position effects — The apparatus should permit shifting or reversal of the gradient within the device to preclude artifacts due to preferences of fish for a particular location (e.g. ends or middle) in the tank. This is taken care of automatically in shuttleboxes where the temperature is always dynamic.

Adjustment of fish to experimental conditions — When first placed in a gradient tank, fish may either cower in a corner or dash about excitedly. Following this, there generally ensues a period of exploratory activity during which the swimming movements of the fish appear "normal." It is during this period that testing usually occurs, generally within the first few days. The exploratory behavior ensures that all available temperature choices are sampled, and so is desirable; it also helps the fish learn to negotiate barriers and apertures. During this period social behavior is generally at a minimum; later, territoriality and dominance interactions in groups tend to increase. In an electronic shuttlebox or a conventional gradient, 24 hours is generally sufficient to permit gravitation to the final preferendum and to allow the fish to become acclimated to it, since upward acclimation tends to be rapid; this can be confirmed by stability of the preferendum over a period of time (about 2 days).

Observation intervals — Observation intervals should be sufficiently spaced to ensure statistical independence of consecutive measurements; this depends on the activity level of the fish (average interval between movements).

Statistics — Statistical reporting of data should be as complete as possible (cf. Reynolds and Casterlin 1976a), preferably including the entire frequency distribution to show skewness. The mode best describes the preferred temperature as that temperature occupied most frequently (modal preferendum). It is easily determined by inspection, and best indicates day–night shifts. It is less sensitive to extreme values than is the mean. Other frequently used measurements include the median, interquartile range, midpoint, range, or upper and lower turnaround or avoidance temperatures. Standard error or standard deviation of the mean may be informative, but the distribution is not often normal. Some measure of skewness is useful, such as Pearson's coefficient [(mean − mode)/standard deviation]; see also Ivlev and Leizerovich (1960) for another measure. If pooling of day and night data gives a bimodal distribution, thermoperiodism is indicated, in which case the mean of the pooled day and night data can be used, or separate statistics for day and night can be given (see Reynolds and Casterlin 1976a). Linear regressions are not recommended to represent the curvilinear relationship between preference and acclimation temperatures.

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pH Activity Curves

THE presence of two allelic LDH (lactate dehydrogenase, E.C.1.1.1.27) subunits in the liver of rainbow trout (*Salmo gairdneri*) was reported by Williscroft and Tsuyuki (1970). In a subsequent study (Tsuyuki and Williscroft 1973), these workers described the pH activity curve of the LDH homotetramers which they designated B_4' and B_4''. Both activity curves had two peaks, an extremely narrow optimum in the physiological pH range, and a broader optimum in the higher pH range. They found the B_4'' optimum (pH 7.302) was lower than the B_4' (pH 7.430) and that the B_4'' conversion rate was about 3.6 times that of the B_4' tetramer. They also observed a higher frequency of the B_4'' alleles in rainbow trout from populations that displayed a tendency to swim against the current and suggested that the higher lactate conversion rate of the LDH B_4'' phenotype might confer a superior swimming endurance.

This suggestion, that swimming endurance might be correlated with the LDH phenotype, was intriguing. If this were truly the case it would provide a unique tool for hatchery selection procedures and for selective breeding of the species. With this in mind, we undertook a study of the pH activity curves of the liver LDH of Atlantic salmon (*Salmo salar*), a salmonid species closely related to rainbow trout, and one in which we had observed a mutant allele of the liver LDH subunit.

The kinetics of Atlantic salmon LDH have been reported by Gesser and Sundell (1971). In our present study freshly frozen salmon liver, muscle, and heart tissues were used. Each extract was prepared from the tissue of a single fish, previously checked and shown to be homozygous in its genotype by starch-gel electrophoresis. The enzyme extracts were prepared by homogenizing the tissue 1:9 in pH 7.500, 0.05 M phosphate buffer. The supernatant from the centrifuged homogenate (30 min at 30.000 g, at 0°C) was diluted further to provide an OD change in the reaction mixture at pH 7.5 of about 0.02 OD U/min at 340 nm (with the appearance or disappearance of NADH). This amounted to an overall dilution of about 1:3000 for liver and somewhat less for muscle and heart extracts. A 0.01 M solution of pyruvate and 0.002 M NADH were used to give a final concentration of 33 nm M pyruvate and 6.6 nm M NADH in the final reaction mixture. A 0.05 M phosphate buffer of the desired pH was prepared for each determination by titration with a Corning model 112 digital pH meter, 0.05 M mono- and dibasic phosphate. The pH determinations were repeatable to within 0.005 pH U. The final reaction mixture consisted of 2.7 ml buffer, 0.1 ml enzyme extract, 0.1 ml substrate, and 0.1 ml coenzyme. The reaction was initiated by addition of enzyme and followed in a Photovolt ERA-I, enzyme rate analyzer which prints out the change in OD for a specified time interval with a sensitivity of 0.0002 OD U/15 s. All reactions were done with temperature-controlled cell at 24.8°C.

The pH activity curves found for salmon heart, liver, and muscle LDH are summarized in Fig. 1. These results agree well with those of Gesser and Sundell (1971) but while some 50 determinations were made in the pH 7–8 range at 0.02 pH U intervals, no spike of activity was found in the physiological range to correspond to that reported by Tsuyuki and Williscroft (1973) in trout.

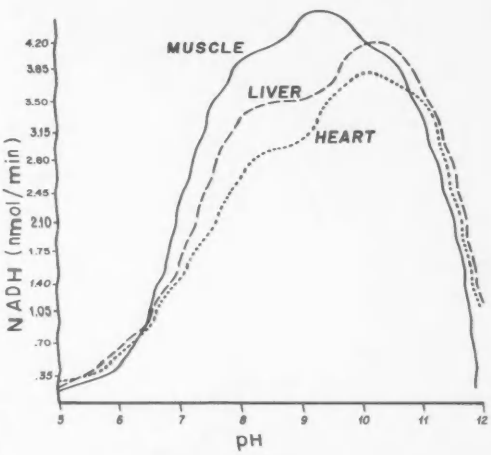

FIG. 1. Effect of pH on activity of Atlantic salmon (*Salmo salar*) muscle, liver, and heart LDH in the lactate to pyruvate direction at 24.8°C. Each curve is a summary of activity determinations at 75 pH values, 50 of which are at approximately 0.02 pH unit intervals between pH 7.00 and 8.100.

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Having failed to demonstrate any salmon liver LDH pH optimum in the physiological range we decided to reexamine the original finding on rainbow trout. Livers from fresh rainbow trout obtained locally and frozen rainbow trout livers of both B_4' and B_4'' homozygous varieties were kindly supplied by Dr Tsuyuki.

The LDH activity of extracts of these livers showed no pH optimum in the pH 7.0–8.0 range. The method described above and Tsuyuki and Williscroft's (1973) method were both carefully followed over this pH range at intervals of about 0.02 pH U. During the runs we noted that mixing the enzyme extract with cofactor as described by Tsuyuki and Williscroft (1973) produced a time-dependent decrease in enzyme activity that was not observed when the reaction was initiated instead by the addition of enzyme extract. When the method of Tsuyuki and Williscroft (1973) was used this potential systematic error was avoided by mixing enzyme and cofactor for only one sample at a time, and by duplicating for each sample the time between premixing of enzyme and cofactor and the initiation of the run with substrate.

We must thus conclude from our results that there is no sharp pH optimum for salmon or rainbow trout liver LDH in the physiological pH range. This is unfortunate since it would be desirable to have such a phenomenon with the pH optimum linked to the LDH genotype and subsequently to the swimming endurance of the fish. However, while our results are negative, the technique is a sensitive one, and we hope other workers will check these pH activity curves in order to fully settle the question of whether or not such sharp pH optima do occur in these species.

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The Coast and Shelf of the Beaufort Sea. *Edited by John C. Reed and John E. Sater. Arctic Institute of North America, Calgary, Alta. 1974. 750 p. $25.*

A symposium was held on January 7 to 9, 1974, under the auspices of the Arctic Institute of North America. It dealt with the coast and shelf of the Beaufort Sea, the objectives being ... "(to) outline the present state of knowledge of the region, to define still existing problems and to interrelate the interests of the various disciplines in a synthesis of Beaufort Sea environments and processes."

As background, it may be noted that a modest amount of study was carried out in the remote and hitherto little-known Beaufort Sea during the 20 or so years immediately following World War II. From about 1968 onward, however, the tempo of research quickened. Various stimuli were responsible. A primary one was the major discovery, in 1968, of oil and gas on the Alaskan north slope. Another was the realization that the Beaufort Sea itself might contain vast oil reserves, which could aid in countering the widespread energy shortages predicted for the not-too-distant future. A further factor has been a growing awareness of the need for harmony between man and his environment — a theme especially germane to the operation of the petroleum industry in the harsh but paradoxically fragile arctic. The proceedings of the symposium, which deal mainly with studies carried out between 1968 and 1973, are a timely reflection of this recent increase in the research effort.

The proceedings comprise more than 35 full papers, abstracts of 5 papers, and several discussions, commentaries, and postsymposium contributions. A group of 4 papers deals with physical oceanography and meteorology. The next 17, the largest number upon any single general topic, treat ice and permafrost. These are succeeded by eight concerned with sedimentation and closely related subjects and about a dozen on chemical and biological themes. The majority of the work is American in origin, and has been carried out in the Alaskan portion of the Beaufort Sea.

Of the physical oceanography papers, the final one — that by O'Rourke — should be read first. It is essentially a review of the state of knowledge in the southeastern (Canadian) part of the Beaufort Sea, being based on studies carried out during the period 1951–73. It provides a useful background against which to consider the entire area. The remaining three papers are more restricted in scope. One implies weak cyclonic patterns of flow at the surface for both air and water in the eastern sea. Another suggests two driving forces for the summer open-water circulation over the Alaskan Beaufort shelf: one, the water intruding, with considerable momentum, from the Bering Sea and giving rise to an eastward flow; and two, the upwelling generated by local easterly winds, resulting in a westerly flow over the eastern half of the shelf. The third paper indicates the predominance of wind-generated effects, such as waves and surges, in controlling the nearshore oceanographic processes important to the coastal geomorphology of northern Alaska. The considerable discussion generated by this relatively small group of contributions emphasized the pressing need for further physical oceanography data from the Beaufort Sea.

The group of papers dealing with ice are concerned primarily with engineering applications to the safe conduct of oil-drilling operations on the continental shelf. It contains the two longest contributions to the symposium. One of these defines three characteristic sea-ice zones of the area (polar-pack, seasonal-pack, and landfast). It then extensively treats both the morphology of the sea ice and the potential for scoring of shelf-bed sediments by the keels associated with the ice. The other paper of comparable length deals exclusively with the ice as a geologic agent on the Alaskan Beaufort shelf. Gouges are found generally to be 0.5–1.0 m deep, and oriented parallel to the shore or to depth contours; the density of occurrence may be as great as 300 or more per kilometer. Ice rafting of sediments away from the coast is believed to be negligible in this area. A further, short, paper also deals with ice scoring on the Alaskan shelf. The scope of these considerations of ice as a geologic factor is new to North American arctic research, as is the emphasis upon engineering considerations in general.

The determination or prediction of pack-ice movement is also considered in several contributions. One deals with the estimation of ice drift by recourse to winds computed, at several points in the Beaufort Sea, from daily surface atmospheric pressures. Another describes one part of a dynamic model for the prediction of movement and a method for testing it. From given barometric-pressure data, the model will represent the physics definable on a 10–100-km length scale with a resolution of 1 day. An elastic plastic representation, in which the ice thickness distribution affects the yield surface, has been chosen as that best approximating the material response.

Two submissions deal with the use of satellite imagery in determining ice movement. One discusses an approach to automated mapping of the movement by statistical examination of infrared data from satellites. Two basic concepts are involved: first, that there is a direct relationship between ice thickness and the radiant temperature at the upper surface of the ice, and second, that the two major ice types — multiyear and first-year — have normal distributions of thickness with differing mean values. Another paper utilizes high-resolution multispectral imagery to study the deformation and drift of ice in the shear area, which is located between the polar pack and the landfast ice.

The landfast ice, the seasonal form which grows over the continental shelf in winter and remains almost immobile until its decay in the following summer, is not neglected; it is the subject of four papers. These cover such aspects as the morphology, breakup dynamics, the tidal- and temperature-related stresses in Alaskan landfast ice, and the general features and extent of Canadian landfast ice. The morphology has been studied by satellite imagery and the breakup was monitored by shore-installed radar.

The crystal fabric and the crushing strength of sea ice are explored in individual papers. Two contributions treat the occurrence and characteristics of subsea permafrost on the Beaufort Shelf. This permafrost is indicated to be widespread, with thicknesses of 30 m or more occurring near shore.

One paper presents some of the problems involved in the monitoring and forecasting of sea ice. This brief but useful submission deals with deficiencies in the various techniques involved (such as use of satellite imagery, aerial reconnaissance, and computer modeling). The author advances what he considers to be a realistic plan for overcoming or circumventing many of these shortcomings. He advocates, for example: greater use of helicopters for ice reconnaissance, as they are less deterred by bad weather than are fixed-wing aircraft; improvement in the cloud-penetrating capability of satellite imagery; and better training of personnel involved in data extraction from this imagery. Another paper describes a system for determining ice movement by means of multiple-path acoustic ranging. The system comprises a self-contained data acquisition and storage unit that measures and records several environmental parameters as well as ice movement. A production model of the system was apparently used successfully in the arctic during the winter of 1972–3.

Of the unabstracted papers on marine sedimentation, one describes the general clay mineralogy and geochemistry of the seabed of the Alaskan Beaufort shelf. The sedimentary processes on the shelves and beaches of northern Alaska are treated in two contributions, while an abstract similarly considers the Yukon coast.

The Colville is the largest Alaskan River draining into the Beaufort sea; however, unlike the much larger Mackenzie River to the east, its flow virtually ceases during the arctic winter. It receives detailed discussion in two aspects: the hydraulic and sedimentary facets of its complex interaction with the Beaufort Sea during spring, the most dynamic period of the year; and the form and other characteristics of the banks enclosing its various distributaries. An abstracted contribution deals with the characteristics of marine gravels found on the Beaufort shelf.

The group of contributions dealing with chemistry and biology treat, to some degree, most elements of the arctic food web. Nutrients are discussed in four papers and primary and secondary production in three. It is suggested that ice algae are of importance to the ecosystem, primarily because of their significant (25–30%) contribution to the annual primary productivity. Alaskan marine birds, beluga and bowhead whales, and polar bears are considered in separate papers. The Arctic cod, a key member of the ecosystem, is the topic of one abstracted paper; it is the only species of fish noted in the symposium. Seals, of which the ringed and bearded species are believed to represent the major menu items in the polar bear diet, are not discussed. In anticipation of increased activity by man in the area, one paper presents the results of a baseline study of mercury content of a variety of environmental materials (plankton, snow, sediments, and river and ocean waters) in the environs of the Alaskan north slope.

The diverse and numerous contributions, with their included bibliographies, made this collection definitive for most research conducted in the Beaufort Sea prior to 1974. The discussions and commentaries provide a valuable source of needs for further research as perceived at the time. With two exceptions, the papers are essentially descriptive in nature, and can be profitably read by the nonspecialist without undue strain. Only the mathematical treatment of the elastic plastic ice model, and that of gouging presented in the long paper on sea-ice morphology, should seriously daunt the "outside" reader.

The symposium has come far toward meeting its ambitious and difficult objectives. The volume has been ably edited; it is free of misprints, and the drawings and photographs are generally extremely clear. The print, on nonglossy paper, while small, is well readable. However, the paperback binding tends quickly to disintegrate even under normal handling of the book.

A postscript of some interest and relevance can be supplied. At about the time the symposium was taking place, Canada's Department of Fisheries and the Environment launched the Beaufort Sea Project. The undertaking consisted of a set of studies on the Beaufort Sea environment. The estimated cost at termination was 12 million dollars, of which about 4.1 million were provided by the petroleum industry. All of the studies were to be essentially completed and evaluated before any offshore exploratory drilling from ships would be permitted on the shelf of the southeastern Beaufort Sea. The main concerns regarding such drilling taking place during the short (3–4-month) open-water season over the shelf were considered to be the following: what damage could be inflicted on the marine environment by a submarine oil well blowout; in what manner this damage could occur; how drilling should be conducted so as to result in minimum damage to the environment; and what threats the environment would pose to the safety and success of drilling operations. This last concern is closely related to the primary thrust of the symposium. The previous three were not treated in the symposium, and thus appear to characterize a logical next step, in both science and morality, for study of the interaction between drilling activity and the environment.

In an attempt to resolve the concerns, the studies undertaken involved a variety of disciplines and of specialized topics, such as: marine biology, marine and shoreline geology; baseline hydrocarbon levels in

the sea; wave, weather, and ice climatology; physical oceanography, including waves, currents, tides, and storm surges; behavior of oil in ice; and oil-spill countermeasures.

Forty-four technical reports were scheduled for completion, and most had been published by August of 1976. One of the reports, No. 39, entitled "A Preliminary Environmental Assessment of Offshore Drilling for Oil in the Beaufort Sea" describes oil well blowout scenarios and summarizes environmental problems associated with exploratory drilling from ships during the summer (open-water) season. In addition, six self-contained "overview" reports entitled "Birds and Mammals," "Fish, Invertebrates and Marine Plants," "The Pathways and Fate of Pollutants," "Geology, Climatology and Prediction," "Oil in Sea Ice," and "Oil Spill Countermeasures" are to be published soon. The Publications are available from the Beaufort Sea Project Office, Department of Fisheries and the Environment, 512–1230 Government Street, Victoria, B.C. V8W 1Y4.

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R69

Survival in Toxic Environments. *Edited by M. A. O. Khan and John P. Bederka Jr. Academic Press, Inc., New York, N.Y., 1974. xxi + 553 p. $19.50.*

This book consists of papers from a symposium organized by the American Society of Zoologists held in Houston, Texas, December 1973. The book is assembled in "rapid manuscript reproduction" form and includes a comprehensive subject index. The material presented addresses itself to chemical environmental pollutants in general, their fate and disposition in the environment, and their bioenvironmental effects. The papers are arranged, in approximately equal number, under five sections: impact on biology of organisms, detoxication mechanisms, role of mixed-function oxidases, recent trends in pesticide research, and nonpesticide pollutants.

The pesticide DDT looms high in most of the papers presented and causes one to think that if there is one word that can most aptly describe the modern age of man it is 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane. Although application of this pesticide is more restrained now than in the past, DDT-related research has a momentum that will continue for some time and probably accounts for its prominence in this book. In one of the papers in the volume, DDT and two of its common metabolites are used in reporting progress on ecosystem modeling of Clear Lake in an attempt to predict the populational and trophodynamic consequences of pesticide use. In another paper evidence is reviewed that documents the environmental impact of various pesticide chemicals (insecticides, herbicides, nematocides, and fungicides) and voices the continuing concern of ecosystem alteration through pesticide use. The impact on marine organisms of xenobiotics of more recent concern (oil, heavy metals, PCBs) is treated in a paper that outlines possible ways of determining sublethal effects of pollutants.

The most interesting part of the book is the three sections devoted generally to the role of metabolism, membrane binding, mixed-function oxidase, genetics, and enzyme induction as detoxification and resistance mechanisms. Herein lies what appears to be the essence of immediate survival in toxic environments. The material presented is impressive not only in the advances made in research and understanding of biotransformation of xenobiotics but in the wonderful capability of living systems to adapt to new environments. It is this capability, however, that presses man to create myriad new chemicals, or increase application of current ones, in his attempt to maintain the *status quo* with his cohabiting pest organisms. The alternative of not doing so, as put by one author in an overview of costs and benefits of pesticides, is for agriculture to become sharecroppers with insects. Curtailment of chemical controls would incur a similar cost to public health. The final section of the book, which largely presents data on the effects of lead and carbon monoxide resulting from automobiles, brings home the issue that the toxic environment in question is very much *our* environment. Hope for an environment of minimal toxicity to humankind is a real concern of the editors.

Most of the papers in this book include a good review of their topic and present research findings in a concise manner with the help of ample tables and figures. The book should appeal to all persons working in the environmental sciences. Chemists and biochemists should find of particular interest the papers detailing methodology and spectral characteristics for P-450 mixed-function oxidase, photoconversion products of cyclodienes, types of biotransformation reactions, and physical–chemical properties of a number of xenobiotics. In company with other papers that illustrate that survival in toxic environments is not just staying alive but is a matter of maintaining the apparent fine juxtaposition of interacting systems in the environment, this volume assembled by Khan and Bederka should be required reading for individuals who ponder the possible jeopardy of man's chemical technology.

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(R79)

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Caloric changes along pulp and paper mill effluent plumes — **M. G. Johnson**
Characterization of suspended particles in some pulp and paper mill effluent plumes — **J. K. Leslie**
Persistence of dissolved organic compounds in kraft pulp and paper mill effluent plumes — **M. E. Fox**
Concentration pattern of chemical constituents in a paper mill's effluent plume: dynamics and model — **J. Polak and M. D. Palmer**
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Relation between Nipigon Bay benthic macroinvertebrates and selected aspects of their habitat — **J. Vander Wal**
Distribution of some organic compounds in the receiving waters of a kraft pulp and paper mill — **B. Brownlee and W. M. J. Strachan**
Distribution of dehydroabietic acid in sediments adjacent to a kraft pulp and paper mill — **B. Brownlee, M. E. Fox, W. M. J. Strachan, and S. R. Joshi**
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Organic contaminant residues in fishes from Nipigon Bay, Lake Superior — **K. L. E. Kaiser**
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Assessment of the acute toxicity, growth impairment, and flesh tainting potential of a bleached kraft mill effluent on rainbow trout (*Salmo gairdneri*) — **D. M. Whittle and K. W. Flood**
Density, distribution, and movement of Nipigon Bay fishes in relation to a pulp and paper mill effluent — **J. R. M. Kelso**

RECENT PUBLICATIONS / RÉCENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed at the end of the section.

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Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études, comprenant deux parties. Les études de 1969, partie 2, incluant le n° 1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

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